NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1982 ANNUAL REPORT

October 1, 1981 through September 30, 1982

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CONTENTS

OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

The Institute has successfully completed its first full year under a reorganization that established four distinct program areas, namely: the Intramural Research Program (IRP); the Toxicology Research and Testing Program (TRTP); the Biometry and Risk Assessment Program (BRAP); and the Extramural Program (EP). The reorganization provides an integrated research response to answer the needs of both the National Toxicology Program and the Institute's commitment to disease prevention through chemical test development and risk assessment.

Activities within the Intramural Research Program continue to provide basic scientific data for the development of programs in disease prevention within population groups and for individuals. Ongoing research in genetic toxicology is concerned with the definition of how hazardous chemicals interact with coding and non-coding regions of DNA with emphasis on cloning and molecular characterization of genes, which will provide the basis for predicting the consequences of somatic and germinal mutations. Cloning of specific genes in Drosophila and the molecular characterization of transposable DNA elements have demonstrated how these elements become inserted and increase mutation rates many fold at gene loci.

Research in reproductive toxicology has developed an animal model for assessing risks from the human transplacental carcinogen diethylstilbestrol (DES). The model predicted the genital abnormality, retained testes, subsequently found in human males and abnormalities of the Fallopian tubes seen in DES-exposed women. The relationship of adenosis and vaginal carcinoma was also shown previously in this work. Study of estrogenic chemicals, using DES as the prototype, continues to examine the toxic products of these substances and how they interact at a molecular level.

In intramural pulmonary studies, tracheal epithelial cells from the rat have been established in culture and the stages of neoplastic transformation caused by chemical carcinogens studied for changes in cell morphology and growth rate. These systems are being evaluated as methods to identify the molecular and genetic changes which accompany neoplastic transformation in pulmonary epithelial cells.

Dichlorobenzidine (DCB), used commerically in the United States primarily in the manufacture of pigment, is known to be a potent carcinogen in animals and is assumed to be carcinogenic in man. Intramural studies have found that elimination of the material into the gastrointestinal tract occurs only by biliary excretion. The gastrointestinal tract, therefore, is the site where hydrolysis and absorption of the substance takes place.

In chemistry, the new technique of fast atom bombardment has extended the range of analytical mass spectrometry to intermediate molecular weight compounds, making it now an even more discriminating and sensitive method for detecting, measuring and separating toxic substances thereby providing advances in toxicological characterization.

The Toxicology Research and Testing Program, an integral component of the National Toxicology Program, develops scientific information about potentially toxic and hazardous chemicals which can be used for protecting the health of people and prevention of chemically-induced disease.

The strategy for test development and validation examines existing and emerging methodologies to identify those which may be adequately sensitive and reproducible. When basic research findings suggest new areas of toxicology testing, TRTP undertakes the appropriate methods development and validation.

A five system testing battery has been implemented for testing mutagenicity of all chemicals selected into the toxicology and carcinogenesis bioassay program.

A bioassay or testing program that originally assessed carcinogenic potential of a chemical in two year tests with rodents has been transformed into a program that also characterizes other major toxic properties of a chemical, such as, the effects on liver, lung, kidney, and fertility. The testing program develops data that permits better assessment of toxic effects that may be related to magnitude and duration of exposure or are peculiar to a given sex or test species or are generally applicable to a group of chemicals within a chemical class. These types of data make the assessment of potential human risk more effective.

Biomathematical simulations aimed at improving the basic experimental design of the two-year bioassay provides information useful for low-dose extrapolation while retaining the power of the bioassay for detecting carcinogenic effects.

Short term in vivo rodent liver carcinogenesis models are being refined to help clarify the nature of carcinogenic responses associated with two year rodent bioassays. A major objective will be to assess the ability of selected chemicals to act as initiators, promoters, or complete carcinogens in these models.

Investigators using the Ames/Salmonella microsome test system, are examining 46 dyes, pigments and colorants for mutagenicity. These substances used extensively in industry have previously not been subjected to this kind of testing.

The Biometry and Risk Assessment Program plans and conducts basic and applied environmental health oriented research in the areas of risk assessment, statistics, biomathematics, and epidemiology.

The program conducts a broad research effort ranging from statistical analysis to biomathematical modeling to develop new or improved methods for quantitative risk estimation, particularly in the areas of carcinogenesis, mutagenesis, and reproduction. Scientists maintain an active research program in statistical methodology relevant to design and analysis issues arising in laboratory experimentation, with special emphasis on toxicological screening assays.

The Institute is continuing epidemiological study on infant feeding. Clinical data on growth, morbidity, and development are being gathered on 900 North Carolina children enrolled in a birth cohort study to investigate any potential adverse effects associated with infant exposure to environmentally-contaminated

breast milk. Results to date indicate a very high prevalence (90 percent) of maternal breast milk contamined with polychlorinated biphenyls and DDE, a metabolite of DDT. This study should lead to development of methodology for investigating low levels of pollutants in humans.

The Extramural Program continues support through Public Health Service grants and awards in those areas essential for meeting the obligations of the Institute. These include toxicology test development, epidemiological studies involving asbestos and other minerals, study of a variety of atmospheric pollutants of increasing importance due to changing energy patterns and investigation of the mechanistic aspects of disease production by environmental agents.

Support will continue for nine Environmental Health Sciences Centers engaged in work on applied and basic research to delineate the role of air pollutants, heavy metals, combustion by-products, industrial compounds and by-products, and physical factors in producing human health disorders, mutations, birth defects and behavioral abnormalities. Support will also continue for Marine and Freshwater Biomedical Research Centers engaged in research using aquatic organisms for testing environmental pollutants and for the development of aquatic models of human environmental disease and disorders.

Within the Extramural Program a major prospective epidemiological study of air pollution and respiratory disease in a six-city investigation is being supported. This study relates respiratory health effects to particulates and sulfur oxides, and features both indoor and outdoor monitoring for SO_2 and NO_2. Preliminary results suggest that both current smokers and those who have never smoked who reside in an area exposed to high levels of photochemical oxidants have poorer lung function than current smokers and people who have never smoked who live in an area exposed to low levels of photochemical oxidants or to high levels of SO_2, particulates, and hydrocarbons.

Competing r & d contracts which support research in the areas of reproductive and developmental toxicology, genetic toxicology, toxicology testing, and effects of toxic agents on the physiology of the immune system have begun or are planned.

Research relating to the acquired deficiency syndrome as a possible etiology in Kaposi's Sarcoma is being supported at one of the Institute's Centers.

In other extramural research, studies in the mouse, as well as in exposed human populations, indicate that elimination of mercury after exposure to methylmercury may be affected by stage of development and diet. The ability of methylmercury to cross the placenta and to be secreted in breast milk are important considerations for hazard evaluation to fetus and the suckling infant.

A new program has been initiated in environmental medicine by the Institute's Extramural Program which encourages submission of research grant proposals for the development of diagnostic technology for patients exposed to hazardous chemicals, especially from waste dumps.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement

During FY 1982 the Office of the Associate Director for Genetics (OADG) continued to fulfill its role in the Genetic Toxicology programs of the Institute by serving as an expert consultant to the Director and the intramural research staff and by developing programs in the areas of genetics and environmental mutagenesis. The OADG has provided a focal point as well as planning and coordination functions in a number of areas of genetic toxicology including (1) international programs, (2) national programs, (3) committees, (4) collaborative studies and (5) collaborative research programs.

International Programs

US-Japan

The Associate Director for Genetics (ADG) is Chairman of The US Panel on Environmental Mutagenesis and Carcinogenesis in the US-Japan Cooperative Medical Science Program. A joint US-Japan Workshop on Genetic Toxicology and Food Mutagens was held at the Lawrence Livermore National Laboratory, Livermore, California, November 5-6, 1981, under the auspices of the US-Japan Environmental Panel. Plans are being made to schedule a conference on Population Monitoring to detect the effects of Environmental Mutagens and Carcinogens to be held in conjunction with the meeting of the Joint Committees in Honolulu on February 7-9, 1983.

ICPEMC

The Associate Director for Genetics attended the meeting of the Executive Board and Commission of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) held in Klingenthal (near Strasbourg) France, November 6-12, 1981. During this period as vice-chairman, the ADG participated in a review of ICPEMC activities including ongoing work of the committees. This review consisted mainly of evaluation of individual working papers prepared for inclusion in the final committee reports.

The ADG also attended the meeting of the Executive Board and Commission of ICPEMC held in Klingenthal, France, April 17-24, 1982. This meeting was devoted to planning of future activities of the Commission in the development of mechanisms to foster scientific interchange and programs in test-method development, validation and utilization in mass-screening programs. The main focus in the Commission meeting was a detailed review and evaluation of the reports of Committees 1 and 3 and Task Groups 4, 5, and 6.

National Programs

EPA Gene-Tox Program

The ADG has participated in several periodically held meetings of the Coordinating Committee during the second phase of the program. The purpose of this phase is for the various Assessment Panels to evaluate the utility of the various test systems, cross-indexing of the data and recommendations of appropriate batteries of tests for mass screening. The Coordinating Committee

reviews the reports of the Panels and reviews the feasibility of panel activities in terms of the computerized data base.

NIEHS Sponsored Workshops

The OADG organized a workshop on the "Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk" which was held at NIEHS on March 28-31, 1982. Participation in the meeting included several NIEHS staff, as well as Foreign and American researchers active in this important area of study. The proceedings of the workshop are in the process of being published.

The ADG, in conjunction with Dr. Ronald Pero of the University of Lund, organized a Joint American-Swedish Workshop on "Individual Susceptibility to Genotoxic Agents in the Human Population", which was held at NIEHS on May 10-12, 1982. The workshop included participation of NIEHS staff and Swedish and American investigators in the area of Epidemiology, Pharmacology, Biochemistry and Genetics. The proceedings are being prepared for publication.

The full report of the Comparative Chemical Mutagenesis Workshop which was organized by the OADG was published in April 1982. The book is edited by the ADG and Dr. M. D. Shelby.

Committees

Subcommittee on Environmental Mutagenesis

The ADG continues to chair the DHHS/CCERP Subcommittee on Environmental Mutagenesis. Due to budgetary and travel restrictions, the number of meetings this FY was limited to three. The topics dealt with amongst others, a review of the NTP program in Genetic Toxicology; an overview of the NAS committee on Chemical Environmental Mutagenesis; a review of NIOSH programs in monitoring High-Risk workers populations; and a review of International Training Program in Environmental Mutagenesis. These and other current issues of importance to government agencies concerned with genetic toxicology will continue to be addressed by the Subcommittee.

At the initiative of the ADG a Committee for Hazard Evaluation and Risk Estimation has been formed at NIEHS to review experimental methodologies and techniques, current literature and recent results, for their suitability for estimation of risk, and to devise recommendations for improving investigational approaches to meet this end. Membership includes staff from BRAP, TRTP and OADG.

Collaborative Studies

WHO - International Program for Chemical Safety

The ADG is chairman of a working group of the International Program for Chemical Safety sponsored by the World Health Organization (WHO), the United Nations Environmental Program (UNEP) and the International Labor Organization (ILO). A meeting of the working group was held in Geneva, Switzerland, November 12-15, 1981 to finalize the choice of chemicals to be used in the study, and the investigators from the member countries who would be asked to participate. The purpose of the program is, on an international scale, to: (1) evaluate in

vitro short-term tests for mutagenesis and carcinogenesis, (2) evaluate short-term in vivo assays for mutagenicity and (3) develope standardized protocols for the performance of short-term tests for mutagenicity during this collaborative study. Letters of invitation have been sent to over 100 investigators and numerous positive responses have thus far been received. Provisions for obtaining and distributing test compounds have been made. It is anticipated that the experimental studies will be underway in early FY 83.

Collaborative Research Programs

Illinois State University

The Principal Investigator continues to submit reports on chemicals tested under the contract. In addition, reports are being received regarding tests to make the mutants recovered homokaryotic as well as reports on more detailed genetic analysis. The data generated during the period that the contract was in force are being utilized as the basis for scientific reports which continue to be prepared for publication.

Public Lectures

F. J. de Serres

1. California Air Resources Board Workshop, University of California, Davis, CA., January 12-14, 1982, "Laboratory Testing - In Vitro Systems".

2. 13th Annual Meeting of Environmental Mutagen Society, Boston, Ma., February 26 - March 1, 1982, "The Role of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC)".

3. Emory University, Atlanta, Ga., March 10, 1982, "Comparison of the Induction of Specific Locus Mutations in Wild-Type and Repair-Deficient Strains of Neurospora crassa".

4. University of Georgia, Athens, Ga., March 12, 1982, "Comparison of the Induction of Specific Locus Mutations in Wild-Type and Repair-Deficient Strains of Neurospora crassa".

William Sheridan

1. 13th Annual Meeting of Environmental Mutagen Society, Boston, Ma., February 26 - March 1, 1982, "Induction by Triethylenemelamine (TEM) of Recessive Lethal Mutations in Post Meiotic Germ Cells of Male Mice".

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

The concern of this office remains the evaluation of human health hazards and in particular from chemicals in man's environment. The focus is not only on newly introduced chemicals by the chemical industries, but also on old, well-established chemicals in the home or the environment, components of food or drink.

It has become well established that the burden of chronic diseases afflicting mankind does not fit into one category, as for instance under carcinogens, but may be composed of many different factors which modify the adverse effects of chemicals either aggravating or accelerating disease processes or on the other hand ameliorating the diseases. The complexity of these situations of human exposure make the health hazard assessment far more difficult and give the public frequently the impression that scientists or administrators do not want to make clear definitive statements or decisions. Education of the public on these difficulties is therefore desirable and this office is trying to help in this task.

Identification of toxicities of chemicals forms the basis of relevant knowledge. However, it represents only the foundation on which additional studies are built concerned with dose-response evaluations, interactions with other chemical or physical agents, genetic susceptibilities, intercurrent diseases and nutritional factors. All of these parameters need to be explored to understand the potential gravity of the situation and the possible actions to be suggested. The interacting factors may contribute to either aggravation or amelioration of the adverse effects.

This office has focused on interactions of chemicals that may alter toxicities based on microsomal or nuclear enzyme induction in target or non-target organs, or that may enhance or inhibit DNA repair processes. It also focuses on interactions that lead to co-carcinogenesis, co-mutagenesis or synergism in teratogenesis, although the underlying mechanisms may not be understood. It has become clear in recent years that parallelism may be found in the mechanisms by which some of the chemicals exert their toxic effects, such as the activation of chemicals to electrophilic substances which can bind instantly to macromolecules as the first detectable effect, but the parallelism may end there and subsequent events need elucidation to understand the processes of carcinogenesis, mutagenesis or teratogenesis in toto, to mention some of the most complex ones.

The recognition of nutritional factors in antagonizing some of the adverse effects has been known for decades but the emphasis of prevention of disease in recent times will possible lead in the right direction. There is a need to carefully prevent overindulgence in vitamin therapy because there is the tendency to believe that more of a good thing is also good.

To help the National Toxicology Program, all members of OHHA contribute suggestions to the NTP Chemical Nomination and Selection Process Committee. Drs. D. B. Walters and B. A. Fowler also participate in recommendation and justification of

nomination of chemicals for bioassays. Dr. Vouk also participates in the work of the NTP Toxicology Design Committee. Similarly OHHA staff contributes in the preparation of the Annual Report on Carcinogens reviewing all data as well as preparing the details on the evidence of carcinogenicity of individual chemicals.

Collaboration with the World Health Organization (WHO) has continued both within the framework of the International Programme on Chemical Safety, and other WHO programs in environmental health, NIEHS being a WHO Collaborating Center on Environmental Health Effects. OHHA has been responsible for NIEHS's contribution to the IPCS program area concerned with comprehensive and short evaluations of priority chemicals; for further work on new WHO guidelines on drinking water quality; and contributed to WHO documents on styrene, titanium, fluorine, and fluorides, principles of health related monitoring for chemical pollutants, and on approaches to estimation of human exposure to air pollutants. As WHO temporary adviser, Dr. Vouk participated in a workshop on methods for estimating exposure, quantifying risk to human health, and measuring chemical injury to ecosystems organized within the framework of IPCS, in Rome, Italy, July 1982. Dr. Falk up-dated his chapter entitled "Modifying Factors, Mechanisms of Potentiation and Antagonism", for publication by WHO in their "Principles and Methods for Evaluating the Toxicity of Chemicals".

The Associate Director remains active as a member of the International Joint Commission's Committee on the Assessment of Health Effects of the Great Lakes Water Quality and is involved in the evaluation of water pollutant chemicals potential health effects on the populations in surrounding counties. He will remain a member of the scientific advisory panel for the Chemical Industry's Institute of Toxicology and as Chairman of the Visiting Committee to Brookhaven National Laboratory but terminate these activities next year as his term expires. His consultantship to Cornell University's program to analyze public policies regarding carcinogens in the chemical industries comparing the U.S.A., the U.K., France, and West Germany has come to an end as did the consultantship to the Office of the Governor, State of North Carolina, in the evaluation of their Chemical Substance Dossiers. He also finished his function on the Ph.D. Advisory Committee at UNC, Chapel Hill, regarding the epidemiological studies of Larry Clark on the dietary level of selenium and vitamin A on skin cancer occurrence in a local population.

The Associate Director of OHHA was invited and participated in a meeting on Possible Mechanisms of Thresholds for Carcinogens and Other Toxic Substances in New York City, where Ms. Jean Bernheim discussed "Interference with DNA Repair by Environmental Chemicals". He also attended as invited speaker a Risk Assessment Symposium in Washington, D. C., disucssing Extrapolation from Animal to Man in Carcinogenesis, and spoke as panel member at the Toxicology Forum meeting in Arlington, Virginia, on "Definitions, Tests, and Correlations of Carcinogens".

PUBLICATIONS

Damstra, T., Jurgelski, W., Jr., Posner, H. S., Vouk, V. B., Bernheim, N. J., Guthrie, J., Luster, M. and Falk, H. L.: The toxicity of PBBs (BP-6 or FF-1) in domestic and laboratory animals. Environ. Hlth. Perspec. 44: 175-188, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20002-10 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Technology Forecasting and Technology Assessment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Warren T. Piver	Chemical Engineer	OHHA NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS
	Herbert S. Posner	Pharmacologist	OHHA NIEHS
	Velimir B. Vouk	Visiting Scientist	OHHA NIEHS
	Jean Bernheim	Microbiologist	OHHA NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this program are to develop techniques for technology forecasting and technology assessment for the chemical process industries that would provide guidance in setting research priorities for environmental chemicals.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: To accomplish these purposes, chemical substances are associated with the method(s) of synthesis or formation, normal commercial use patterns, and methods of disposal. With this approach, it is possible to examine and explain the origin of contaminants and by-products in intermediate and end-product chemicals. Along with these activities that relate to commercialization of the chemical, data are collected on environmental transport and transformation, the potential for bioaccumulation, and the toxicity of the chemical.

Assembling this information on individual chemicals and groups of chemicals accomplishes the following: (1) provides a better understanding of origins of pollutants; (2) identifies gaps in knowledge; (3) identifies specific industrial and commercial processes and operations requiring pollutant monitoring and possible equipment design modifications; (4) identifies trends in rates of production, rates of substitution of products, and the impact of Federal Legislation and programs on chemical development; and (5) provides additional data on relationships between chemical structure and biological activity and environmental effects.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year major attention has been given to waste disposal technologies and methods for chemical selection for mutagenicity testing based on molecular structure and projected biological activity. In the area of waste disposal technology, emphasis has been given to the design of landfills for chemicals, and the design of incinerators for hazardous and toxic chemical wastes. In the development of methods for chemical selection, attention is being given to arranging commercially important chemicals into groups that have similar molecular structures.

In the design of chemical landfills, design criteria have been developed for the saturated zone of soil beneath the landfill that are a function of soil conductivity, adsorptive capacity, and chemical reactivity of individual chemicals. Using a one-dimensional representation of the equation of continuity for this transport system, and assuming constant values for all transport properties, a saturated soil thickness can be estimated. This estimate of soil thickness required to minimize the amount of a chemical entering groundwater is then used in a numerical solution for this same problem that allows for greater variability of soil and transport properties. Based on this analysis, laboratory experiments will be designed to refine the model. Efforts will continue to develop models that predict transport of more than one chemical in two and three spacial dimensions in saturated soils and to simulate transport of solutes in unsaturated soils.

The RCRA requirements for incinerator operation require destruction efficiencies of 99.99% for toxic chemicals. In order to minimize environmental release of toxic chemicals, principles of chemical reactor design are being applied to incinerators. For multi-chemical feed streams, the operations of the incinerator must initially cause the chemical to be in a gaseous or very finely divided state. In this connection, the incineration of chemical wastes is similar to a gas-phase

isothermal plug-flow chemical reactor. The level of chemical destruction is a function of the temperature and length of the isothermal section. Work is continuing to refine the bases for these conclusions and to develop correlations that can be tested in commercially available incinerators. The results of this analysis will form the basis for selection and operation of an incinerator for Institute laboratory wastes and for the development of general principles of incinerator design for toxic chemicals.

The selection of commercially important chemicals for mutagenicity testing is an NTP activity. Efforts so far have been towards identification of groups of chemicals that are similar to chemicals with demonstrated biological activity. At the present time, 600 individual chemicals have been recommended for mutagenicity screening in the different salmonella in vitro test systems. This work will continue because not all of the commercially important chemicals have been examined. In addition, results from the mutagenicity screening program are being examined and groups of chemicals with similar molecular structures are being identified. Based on the examination of results, commercially important chemicals will not only be added to these groups but will be recommended for testing in the mutagenicity screening program. Another activity has been to examine the combustion of coal as a major source of trace metal emissions to the atmosphere and to examine methods of determination of mutagenicity of elements. The salmonella test system is not a good system for metals and recommendations will be made to the NTP that other test systems such as the "Rec" assay and the "Infidelity of DNA synthesis" be developed for this purpose.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These activities will continue to provide necessary information about the entry of commercially important chemicals into the environment and identify gaps in knowledge with regard to toxicity, bioaccumulation, and environmental transport and transformation. Such information provides a firmer justification for toxicity evaluation programs within the Institute, and facilitates the performance of duties as a member of the NTP Chemical Selection Committee.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20003-09 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Preventive Surveillance of Environmental Chemicals for Toxic Potential

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
OTHER:	Raymond E. Shapiro	Asst. Dir. Toxicol. Coord.	OD	NIEHS
	Philip J. Landrigan	Dir., Div. of Surveillance, Hazard Evaluation and Field Studies	NIOSH	CDC
	OHHA Staff		OHHA	NIEHS

COOPERATING UNITS (if any)
CDC, NIOSH, Cincinnati Laboratories

LAB/BRANCH
OD

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Areas that are being followed or were reviewed include those of methanol (proposed for many new and expanded uses), paraquat (an herbicide also used for destructive spraying of illicit marihuana), conditions causing stratospheric alterations (particularly the presumed ozone reduction and transmission of additional short wave solar ultraviolet radiation to earth), aminimide compounds, hazards of synthetic energy production processes (mainly coal conversion), asbestos substitutes, polybrominated biphenyls, lead toxicity, and safety testing of aspartame.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, consultations with appropriate individuals, consideration of structure-activity relationships, preparation of reports, discussions with those who might assist in further laboratory or theoretical considerations, and preparation of manuscripts for publication; reviews of documents and manuscripts and response to letters as requested.

MAJOR FINDINGS AND PROPOSED COURSE:

Methanol: The toxicities of methanol, ethanol, isopropanol, and gasoline to humans were compared. Pneumonitis, mainly in children, resulting from aspiration into the lungs is the most serious usual hazard of unintended swallowing of gasoline. In uncomplicated cases, bedrest and supportive care are sufficient for recovery. Inhalation in confined locations has caused death believed to be by sedation while sniffing or huffing (mouth breathing) are believed to have caused death by cardiac toxicity. Alkyl lead has been an additional inhalational hazard when present in the gasoline.

Between methanol, ethanol and isopropanol, methanol has the highest vapor pressure, the lowest minimal oral lethal dose, the highest rate of skin absorption, the slowest metabolism and the longest latent period before symptoms. Low concentrations of methanol in gasoline and low concentrations of gasoline in methanol result in 3-4-fold faster skin absorption of the methanol. Methanol, and less so isopropanol, has been an abuse problem in place of ethanol. In general, treatment for methanol poisoning has been the most intense and prolonged. Methanol and isopropanol can potentiate the toxicity of some other chemicals, as known more extensively for ethanol potentiations. Triage was recently utilized to cope with an incident of about 50 cases of methanol poisoning and additional suspected cases.

Paraquat: Reports continue to appear concerning paraquat toxicity in humans and experimental animals. Most reports about interactions from co-exposure to other chemicals indicate increased toxicity. Ascorbic acid appears to increase rather than decrease the toxicity of paraquat. Single reports suggest beneficial effects of niacin in rats and of colchicine in a patient; these require further study.

Small but reproducible amounts of paraquat contamination on marihuana were shown, by research on contract, to come through in the mainstream smoke, while a large loss occurred via the side-stream smoke. Smoking characteristics for the marihuana were rather different from those with tobacco smoking and, therefore, the latter only approximate the former. The marketer of paraquat preparations in the U. S. requested that paraquat not be used to spray marihuana. The use, they contend, would be inconsistent with the label to the extent that, though marihuana uses are generally illegal, marihauna was intended as a crop. They also note that, were legal action to occur, they would not consider themselves to be liable because of the non-authorized used.

Stratospheric modifications: A study by the National Academy of Sciences suggests that reduction of stratospheric ozone following release of some of the fluorocarbons, particularly F-11 and F-12, would now be about half that which they

calculated the last time this was done. However, the incidence of skin cancers are now estimated to be about twice that previously estimated, resulting in little overall change in this clinical parameter. EPA has recently taken the position that any further regulation in the U. S. would await direct evidence that a decrease of stratospheric ozone has occurred, rather than utilizing related chemical and computer-modeling data.

Aminimide compounds: Additional patents have been granted on the bactericidal and fungicidal activities of long chain fatty acid aminimide congeners. However, there still remains little data in the open literature on safety testing and none on metabolism of aminimide compounds.

Uses of aminimides have included incorporation into adhesive preparations, as surfactants for a wide variety of purposes, in electroplating and as plant growth inhibitors. The commercialization of a particular series of congeners, based upon use of 1,1-dimethylhydrazine during synthesis, was said to have been constrained in the U. S. due to the closing of the only remaining plant preparing it via dimethylnitrosamine, which is a potent carcinogen. As a result, alternate routes for commercial synthesis of 1,1-dimethylhydrazine, also a carcinogen, are being sought.

Lead: Recent literature relating to potentially toxic effects of low concentrations of lead from gasoline were reveiwed for DHHS's input at a hearing sponsored by EPA. It was indicated that particularly effects on the nervous system are still being identified at lower concentrations of lead in blood, formerly felt to be innocuous. Slightly increased concentrations of lead in blood are also associated with decreased concentrations of the hormonal form of Vitamin D, that is, 1,25-dihydroxyvitamin D.

Three practices that affect subpopulations have increased lead intake and resulted in symptomology. These are the "mouthing" of soil and dust mainly by 1-4 year olds, and sniffing or huffing (mouth breathing) of leaded gasoline by older children and young adults for the odor, sedative effect or psychotropic effects of the vapor.

Aspartame: Health-related issues concerning this newly approved artificial sweetener are being considered and the literature followed.

Other areas being followed: Some other areas being followed include: coal conversion products, asbestos, substitutes for asbestos and polybrominated biphenyls. Documents are reviewed for their health hazard aspects as requested and requests for information are answered.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas where research is needed in order to prevent or reduce toxicologic hazard from environmental chemicals or physical agents. It is primarily a program of preventive and early-warning surveillance. It is linked closely to research dealing with the scope of the problem, the mechanisms involved, and preventive measures. It also considers potential means of diagnosis and therapeutics where these are possible.

PUBLICATIONS

Posner, H. S.: Letter to the editor. (response to letter concerning nuclear waste disposal, age of the earth and "scientific creationism"). Environ. Hlth. Perspec. 43: 169, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20007-05 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Identification and Evaluation of Environmental Health Hazards: Chemicals and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS

COOPERATING UNITS (if any)
Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The objective of this project is to identify and evaluate real and potential health hazards in the environment with emphasis on chemicals both as toxicants and as carcinogens.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Review of existing data in the open literature and in unpublished manuscripts; preparation of reports and monographs; consultation with scientists from other governmental agencies, industry, and academia, both domestic and foreign.

MAJOR FINDINGS AND PROPOSED COURSE: 1. The Principal Investigator was a member of the Subcommittee for the NTP Chemical Nomination and Selection Process Committee.

2. The Principal Investigator completed a review of caffeine as an environmental agent. The new information available did not warrant publication of a review on toxicity of the chemical as initially projected. A summary for internal use was prepared, however.

3. The Principal Investigator has begun a review of the chemicals in use in the microelectronics industry, based on several recent studies of safety in the industry by diverse groups including the State of California Department of Industrial Relations, Division of Occupational Safety and Health; the Semiconductor Industry Association; and the National Institute of Occupational Safety and Health. A partial list of chemicals to which workers have the highest potential exposure during silicon device manufacturing and gallium arsenide semiconductor fabrication has been submitted to the above committee. This list will be updated and expanded as new information becomes available. In addition, a list of liquid crystals and associated chemicals used in the production of light emitting iodes is in preparation.

4. The Principal Investigator is exploring the value of a possible review of the known toxic properties with identification of priorities for further toxicological studies.

5. A review in collaboration with Drs. Robert Dixon and Robert Pratt, tentatively titled: "Chemical Toxicity *in Utero*: A Review of Targets, Mechanisms and Methods has been initiated by the Principal Investigator.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Critical reviews and periodic re-evaluations of the type described provide (1) a basis for a more balanced assessment of the risks vs. benefits associated with potential and existing health hazards and (2) reveal those deficiencies and inconsistencies in the available scientific information which require further research and new approaches.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20008-05 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William Jurgelski, Jr.	Medical Officer	OHHA NIEHS
OTHER:	Pearlie M. Hudson	Physical Sci. Tech.	LET NIEHS
	Hans L. Falk	Assoc. Dir. for OHHA	OHHA NIEHS
	L. E. Zimmerman	Ophthalmic Pathologist	AFIP
	J. M. Henry	Neuropathologist	AFIP
	N. Palmer	Renal Pathologist	The Wilm's Tumor Study Group, Ohio State Univ.
	S. Hoffman	Oral Pathologist	Univ. Alabama
	L. J. Rubenstein	Neuropathologist	Univ. Virginia
	M. Herman	Neuropathologist	Univ. Virginia

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Health Hazard Assessment

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to develop the opossum Didelphis virginiana as a biomedical model by (1) characterizing the normal neonatal and developing opossum anatomically and physiologically and (2) determining the pathophysiologic response of these animals to selected environmental toxins and carcinogens.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Collaborative Studies

MAJOR FINDINGS AND PROPOSED COURSE:

1. Invited Papers
   a. An invited review entitled "Marsupials as Animals for Biomedical Research" has been published in the Comparative Pathology Bulletin 13: 1, 5-6, 1981.
   b. An invited review entitled "An Alternative Animal Model for Perinatal Carcinogenesis" has been accepted for publication in Biological Research in Pregnancy 3: No. 4, 1982.
   c. An invited review entitled "The Marsupial as an Animal Model in Space Biology" is in preparation for Biological Reviews.

2. Collaborative Studies and Consultation. The principle investigator is collaborating in several studies, utilizing the opossum, being conducted or planned at other institutions.

   (a) In collaboration with Dr. Bernd Hamprecht of the Max Planck Institute of Biochemistry in Munich, West Germany, a small opossum breeding colony has been successfully established with the objective of reproducing the brain (ganglioglioma) and eye (teratoid medullo-epithelioma) neoplasms induced with ethyl nitrosourea. (Please see Annual Reports for FY 74 and 75). Tissue from these two tumor types will be (a) frozen as part of a tumor tissue bank, (b) innoculated into nude mice, and (c) placed in tissue culture for morphological, biochemical, and electrophysiological studies of neoplastic neurons in vitro.

   (b) The Principal Investigator functions as a consultant to the Laboratory of Gastrointestinal Physiopathology, Department of Medicine, University of Louvain, Belgium, (Drs. J. Jaspens and G. Vantrappen) in a study of the physiology of peristaltic contraction in the esophagus of the opossum.

   (c) The Principal Investigator is collaborating in establishing a research program to reproduce embryonal tumors of the jaw using the opossum neonate by the Department of Pathology, University of Alabama Medical School, Birmingham, Alabama (Dr. S. Hoffman).

   (d) The Principal Investigator is collaborating in establishing a research project involving the induction of retinoblastic neoplasms in the neonatal opossum at the Eye Institute of Retina Foundation, Harvard Medical School (Dr. Mukai).

   (e) The Principal Investigator is collaborating in a study of divergent neoplastic neural differentiation and the early expression of glial and neuronal cell markers being conducted by Dr. L. J. Rubinstein and M. Herman of The Department of Pathology, University of Virginia.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marsupial model provides an opportunity to directly evaluate the relationship between susceptibility to embryonal carcinogenesis and differentiation of the target tissue. The model may also be of value in exploring the apparent interrelationship among oncogenesis, teratogenesis, and mutagenesis in the absence of the major handicap intrinsic to the eutherian animals; namely, the impossibility of distinguishing direct carcinogen induced teratologic/mutagenic changes from identical lesions which are the indirect result of adverse physical and physiologic effects of the carcinogen on the maternal and fetoplacental unit. In the semi-embryonic, semi-fetal opossum orally or parenterally exposed to a carcinogen, mutations and malformations can only be either carcinogen induced or spontaneous.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20009-04 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Identification of Potential Environmental Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jean Bernheim	Microbiologist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim is to identify and evaluate potential health hazards in the environment from exposure to chemicals by an in-depth search of the literature. The mechanism of action of the toxicant is the ultimate goal of this endeavor. Activities include compiling toxicological data for inclusion in data base records and profiles of chemicals identified in waste dumps throughout the United States; and collecting and documenting carcinogenicity information for the NTP Annual Reports of Carcinogens. Other areas of interest include chemical/chemical interactions and chromosome breakage and/or sister chromatid exchange in human cells.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The open literature is consulted (computer searches, library, personal contacts) for identification of chemicals that are associated with certain biological or toxicological effects. This information is collected, classified on the basis of mechanism of action, chemical structure, toxicity, etc., and is completely documented. The report may be prepared for use as background documentation or serve the staff of OHHA in their function.

MAJOR FINDINGS AND PROPOSED COURSE:
Chemical Waste Dumps (Love Canal): A tabularization of the available toxicity data was compiled for the 93 compounds in the Love Canal "Declaration Area". Of these compounds 37 were new additions to the original list of chemicals found in Love Canal (1/30/80). This information was presented to the Director's office for use at the "Research Needs for Evaluation of Health Effects of Toxic Chemical Waste Dumps" meeting held at NIEHS, October 27-28, 1981. Toxicological data profiles will be compiled on chemicals known to be in the "priority" EPA superfund waste dump sites.

Carcinogenicity data for NTP's Annual Reports on Carcinogens: Carcinogenicity data were collected, documented, and written for inclusion in the National Toxicology Program's Annual Reports on Carcinogens. There were 88 compounds in the Second Annual Report on Carcinogens published December 1981, and it was expanded by 28 compounds for the Third Annual Report on Carcinogens to be published in the Fall of 1982.

Human chromosome breakage and/or sister chromatid exchange: The literature contains information on that topic which needs clarification and assessment. Different types of chromosomal abnormalities and specific chemical exposures have been searched for, as well as their relationship to mutagenicity and/or carcinogenicity.

Chemical/Chemical Interactions: This is a long-term project which has been initiated because of the multiple nature of most chemical exposures to the human population. An open-ended file has been started to collect specific types of interations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Information available in the literature often times can give us clues on potential hazards that may be anticipated on exposure to new chemicals which are bearing close relationships to other better known chemicals. The numbers of chemicals which exist including those introduced each year as new compounds necessitates novel and anticipatory approaches to the selection of chemicals for testing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20010-02 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Identification and mechanism of action of DNA repair inhibitors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jean Bernheim Microbiologist OHHA NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4		0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim is to identify and evaluate potential health hazards in the environment from exposure to chemicals referred to as DNA repair inhibitors by an in-depth search of the literature. This includes a comprehensive study of the area of interference of DNA repair by chemical, physical, and genetic factors. The mechanism of action of the toxicant is the ultimate goal of this endeavor and to discover if specific mechanisms exist for DNA repair inhibition and whether this information can be practically applied.

PROJECT DESCRIPTION

METHODS EMPLOYED: A major resource has been an open-ended file developed over three years pertaining to chemical interactions, DNA repair and its inhibition and other pertinent subjects. The open literature is consulted (via computer seraches, personal communications, and continuous monitoring of the most recent issues of relevant journals) and this information is classified, analyzed, and incorporated into reports.

MAJOR FINDINGS AND PROPOSED COURSE: DNA is vulnerable to physical and chemical attack, but effective enzymatic mechanisms for repairing such damage are present in procaryotes and eucaryotes. Cells genetically defective in a particular repair pathway are more easily killed, mutated or transformed by radiation or chemicals. However, even in the genetically non-defective cell DNA repair may not be as efficient as required and lack of DNA repair capacity was found to be due to some chemical insults to the systems.

The purpose of this project is to provide a comprehensive overview of the literature pertaining to the interference of DNA repair by chemical, physical, and genetic factors. By what mechanism(s) do they function? One promising and current approach has been to look for the existence of co-mutagens leading to synergism in the mutagenic parameters due to the presence of DNA repair inhibitors. The questions to be answered are: Do they all represent a non-specific, suppressive effect on DNA synthesis or some other related function or are there "true" DNA repair inhibitors with a specific, selective effect? What relationship does DNA repair inhibition have to chromosomal aberration production and/or carcinogenicity? What is the potential of studying DNA repair inhibition as an important new area for the investigation of toxicologic parameters of chemical agents? To what practical use can this knowledge be applied, i.e., in chemotherapy and as radiosensitizers in radiation therapy of cancer?

A forty-minute synopsis of this information was presented at the "Cancer and the Environment Symposium-Possible mechanisms of thresholds for carcinogens and other toxic substances" sponsored by the International Study Center for Environmental Health Sciences (In New York City on November 2, 1981).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information available in the literature often times can give us clues on potential hazards that may be anticipated on exposure to new chemicals which are bearing close relationship to other better known chemicals either by structure or function. DNA repair inhibition has already demonstrated usefulness in increasing the beneficial effects of chemotherapy. However, it may also prove detrimental in its synergistic action with mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20011-02 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Comprehensive Evaluations of Biological Effects of Chemicals and Health Hazard Assessment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Velimir B. Vouk	Visiting Scientist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Warren T. Piver	Chemical Engineer	OHHA	NIEHS
	Janet Guthrie	Microbiologist	OHHA	NIEHS
	Laila Moustafa	Scientist	IRRU*	WHO

*WHO Interregional Research Unit located at NIEHS

COOPERATING UNITS (if any)
Institute-wide and some 20 research units in the USA and other countries

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this long-term project, carried out partly within the framework of WHO/UNEP/ILO International Programme on Chemical Safety (IPCS), is to prepare evaluations of biological effects and health hazard assessments of selected chemicals, including evaluation of toxicological methods on which published data are based. Focus has been on the assessment of 2,6-toluene diamine, phthalic acid esters, and selected metals and metal compounds. The methodological component of the project dealt with methods for assessing the effects of chemicals on reproductive function, methods for estimating exposure and quantifying risk to human health, biological tests in the evaluation of mutagenicity and carcinogenicity of air pollutants, and principles and methods for toxicity evaluation of chemicals.

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, reviewing and selecting relevant publications, preparation of data files which are continuously updated; consultation with appropriate individual scientists; preparation of draft environmental health criteria and similar documents, including methodology, which are then reviewed and revised by international groups of experts; scientific editing of documents approved by international groups of experts.

MAJOR FINDINGS AND PROPOSED COURSE:

2,6-Toluene diamine: This chemical is a by-product of the synthesis of 2,4-toluene diamine. Mixtures of 2,6-toluene diamine and 2,4-toluene diamine are used in the synthesis of toluene diisocyanate which is the major isocyanate applied in the production of polyurethane foams and elastomers. 2,6-Toluene diamine is also used as an intermediate in the production of dyes for furs and textiles. Since 2,4- and 2,6-isomers occur mostly as mixtures, they have to be evaluated together. 2,4-diaminotoluene is carcinogenic in rats but the 2,6-isomer was not found to be carcinogenic either in rats or in mice. A draft of the evaluation document has been prepared in collaboration with WHO IRRU and transmitted for review and revision to national focal points for the International Programme on Chemical Safety (IPCS).

Phthalic acid esters: These plasticizers are produced in large volumes, and seem to be rather widely distributed in the environment. A NTP bioassay report indicated that di(2-ethyl hexyl) phthalate (DEHP) is carcinogenic in Fischer 344 and B6C3F1 mice. This has revived national and international interest in phthalic acid esters as environmental pollutants. The results of a recent NTP bioassay of diallylphthalate with B6C3F1/N mice do not indicate that this compound, widely used as crosslinking agent for unsaturated polyesters, is carcinogenic in this strain of mice. A carcinogenicity study by NTP of the same compound in Fisher 344/N rats is still in progress. A draft environmental health criteria document on phthalic acid esters has been prepared in collaboration with WHO IRRU and sent for comments to the national focal points for IPCS.

Toxicology of metals: Dr. Vouk is one of the editors, together with Dr. Lars Friberg and Dr. Gunnar Nordberg from Sweden of the Handbook on the Toxicology of Metals, published in 1979 by Elsevier/North Holland Biomedical Press, Amsterdam. The Handbook was reprinted with corrections in 1980. The second edition is now in preparation. Dr. Vouk is the author and/or co-author of 10 chapters of this monograph, which consist of a general part and a special part containing critical reviews of the toxicology of some 30 metals and their compounds. Other NIEHS participants in this project are Dr. Robert A. Goyer and Dr. Bruce A. Fowler.

Principles and methods for evaluation of the toxicity of chemicals: A valid international evaluation and health hazard assessment of chemicals depends on the comparability of results obtained in different laboratories nationwide and

worldwide, and this in turn depends on the understanding of principles, approaches, and methods used for toxicity testing. With this in mind, WHO published in 1978 the first volume of a monograph on principles and methods for evaluating the toxicity of chemicals (Environmental Health Criteria 6, UNEP/WHO, Geneva). The manuscript of the second volume of this monograph is being edited and updated. Chapters on methods for testing selected organ system functions; effects on reproduction, embryo- and fetotoxicity; neurological and behavioral effects; effect on skin and the eye; cumulation and adaptation; and modifying factors have been completed and sent for review and updating, where necessary, to the scientists participating in this IPCS project.

Biological tests in the evaluation of mutagenicity and carcinogenicity of air pollutants: This international study was undertaken by the Karolinska Institute, Stockholm, Sweden, in collaboration with the National Institute of Environmental Medicine and the National Board of Occupational Health and Safety, under the sponsorship of the Swedish Government Committees on Automotive Air Pollution and on Coal, Health and Environment. The objective was to examine the relevance of short-term and long-term biological tests for mutagenicity to the assessment of human carcinogenic risk that may arise from exposure to air pollution from motor vehicle exhausts and coal combustion products. Drs. Vouk and Piver addressed the question of metallic elements in fossil fuel combusion products. Dr. Vouk attended the meeting held in Stockholm, February 8-12, 1982, and participated in the drafting of a joint report prepared by about 40 scientist from different countries.

Methods for Assessing the Effects of Chemicals on Reproductive Functions: Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) has been established in 1980 within the framework of IPCS, under the sponsorship of WHO, the International Council of Scientific Unions/Scientific Committee on the Problems of the Environment (ICSU/SCOPE), and the United Nations Environment Programme (UNEP). The objective of SGOMSEC is to examine methods for the predictive evaluation of the adverse effects of chemicals on human subjects and other forms of life. The first study that SGOMSEC undertook was concerned with methods for the assessment of the effects of chemicals on reproductive functions of mammals, vertebrates other than mammals, invertebrates, higher plants, algae and microorganisms. Dr. Vouk participated in the workshop at Ispra, Italy, in May 1981, at which this study was completed and edited the joint report of the meeting.

Methods for estimating exposure, quantifying risk to human health, and measuring chemical injury to ecosystems: This is the second SGOMSEC study. The workshop was held in Rome, in July 1982. As member of SGOMSEC, Dr. Vouk participated in the workshop and chaired a working group on laboratory models and methods for obtaining quantitative data on mutagenesis and carcinogenesis. The whole project on quantitative estimation of risk to human health was directed by Dr. David Hoel, Director BRAP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An international evaluation of biological effects of selected chemicals and of methods used to obtain toxicological data will enable the Institute to review and, if necessary, reorient some of its research efforts towards objectives which are of particular scientific interest nationwide and worldwide.

PUBLICATIONS

Vouk, V. B. and Piver, W. T.: Metallic elements in fossil fuel combustion products. Amounts and form. Evaluation of carcinogenicity and mutagenicity. Environ. Hlth. Perspec. (in press).

Vouk, V. B. and Sheehan, P. J.: Methods for Assessing the Effects of Chemicals on Reproductive Functions. SCOPE 22. Chichester, New York, Brisbane, Toronto, John Wiley and Sons, 1982 (in press).

Vouk, V. B., Ozolins, G., Hasegawa, Y. and Parizek, J.: Some international activities in environmental health monitoring and surveillance. Environ. Monit. Assess. 17, 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20013-01 OHHA

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Abstracts on Select Toxicological Issues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Guthrie Microbiologist OHHA NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Abstraction and interpretation of pertinent research data from the medical and toxicological literature on issues that are to be dealt with in the Office of Health Hazard Assessment. Recent topics of concern were as follows: Co-oxidation of xenotoxic chemicals by prostaglandin synthetase, the nature of cytosolic receptors for steroids and other non-related chemicals, the abundance of multiple forms of cytochrome P-450 and their significance in metabolism of xenobiotics, virus - chemical interactions in relation to the development of cancer, the toxic effects of breakdown products of some non-persistent pesticides, etc.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Literature searches using computer data bases; interviews and correspondence with researchers in the field.

MAJOR FINDINGS AND PROPOSED COURSE: Interpretation of findings in the literature helps this office to better assess health hazards. In the future, these projects will continue and be enlarged as the need arises.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: By keeping abreast of new developments and issues in toxicology, this office can make better informed recommendations to institute scientists and other interested parties about environmental and occupational health hazards due to chemical, physical and biological agents.

BATTELLE PACIFIC NORTHWEST LABORATORIES - Richland, Washington
(NIH-NIEHS-78-2150)

TITLE: Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $116,990

PROJECT DESCRIPTION

OBJECTIVES: The objectives of these investigations are to determine using advanced radiotracer, spectroscopic, and chromatographic techniques: (1) the rate and extent of uptake of metals from soils by representative broadleafed plants; (2) the translocation of metals in plants; (3) the effect of metals on soil microbiota and soil microbial processes; (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues; and (5) the potential for soil formation of organometal complexes, and subsequent uptake of these compounds by plants. Implicit in these objectives are the development of suitable methods for determining the chemical forms of metals in soils, plants, and microbial tissues.

The metals are nickel, cadmium, chromium, and thallium, and the plants are soybeans.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light; 10 hr dark), temperature (27°C light' 20°C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 and 142 days from planting). Plants were separated by roots, stems, leaves, immature bean pods, mature bean pods, and mature beans. In all cases the tissues were analyzed for total metal radioisotopes. In addition, uptake kinetic studies were performed and isotherms determined for both single and multiple element uptake.

In soil biochemical studies, enrichment techniques have been used to select for microorganisms that exhibit a high resistance to added metal concentrations. Procedures employing TLC, column chromatography, electrophoresis, and mass spectrometry are being developed to identify the metabolites of this resistance and/or detoxication process. These same analytical procedures are being used to characterize the chemical form of the metal in plant xylem, leaves, stems, and fruit.

In order to separate neutral organometallic complexes that had been made by the soil microbes, the total metal complex extract passed through a soil column. Charged complexes were adsorbed to humic and fulvic acid fragments of the soil, and neutral complexes which will most likely be transported to the root membrane passed through. The chemical characteristics of these neutral organometallic complexes were then determined by GC/MS.

MAJOR FINDINGS AND PROPOSED COURSE: From earlier studies, it had been demonstrated that soil microbes were capable of converting the inorganic metal salt into a series of organometallic complexes. In terms of mobility in soil, solution and uptake by plants, however, the complexes of most importance are the electronically neutral ones. Uptake kinetic studies with soybeans using nickel and cadmium have been completed. Nickel absorption, measured as a function of concentration, demonstrated the presence of multiple absorption isotherms, each of which conform to Michaelis-Menten kinetics. Absorption of Ni^{+2} was inhibited by Cu^{+2} and Zn^{+2} indicating that the three ions may be absorbed by the same carrier site. Cadmium absorption also exhibited a multiple absorption isotherm behavior. Cd^{+2} absorption was competitively inhibited by Cu^{+2}, Fe^{+2}, Mn^{+2}, and Zn^{+2}, again suggesting a common carrier site for these five ions. The nature of the complexes of these elements in plants appears to be composed of asparagine and glutamine in a ratio of 3:1.

Distribution studies for nickel and cadmium in soybean plants have been completed. At senescence, >70% of Ni present in the stem was remobilized to the seeds. Nickel accumulated in seeds was primarily associated with the cotyledons. In the cotyledons, 80% was associated with the soluble whey fraction and 70% of this fraction was composed of Ni-containing components with MW <10,000. The behavior of Cd^{+2} was much different from Ni^{+2}. Cadmium was strongly retained by the roots after absorption. Of the tissues examined, cadmium was found mostly in the soluble fraction with >80% being associated with components >10,000 MW. The cadmium that does reach the seeds was associated with the soy whey of cotyledons. Studies are continuing to determine the amino acids that comprise the metal complexes and to study the induction of metallothionein by cadmium. Studies are also in process to determine the distribution of the four metals in other vegetables, such as bush beans and squash. Studies are in process to simulate the movement of metals in soils and plants. The experimental data will be used to calibrate the model so that it can be used as a predictive tool.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides or as contaminants of fertilizers. The importance of soil and plants in this pathway is the conversion of the metal in the soil to more soluble and more toxic complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. In related animal feeding studies with plutonium, it has been demonstrated that the rate of absorption from the GI tract for the organometallic complexes of plutonium in plants was much greater than for inorganic plutonium salts.

INTERNATIONAL PROGRAMS

INTERNATIONAL PROGRAMS
OFFICE OF THE ASSISTANT TO THE DIRECTOR FOR INTERNATIONAL AFFAIRS
Summary Statement

The Assistant to the Director for International Affairs is responsible for the following program areas:

US-China Cooperation

Cooperation between the United States and the People's Republic of China in the area of environmental health was initiated during 1980 under the US-PRC Agreement for Cooperation in the Science and Technology of Medicine and Public Health. NIEHS is a participant in the topic on public health and health services research, which includes concerns relating to environmental and occupational health. Exploratory discussions between both sides have been held during exchange visits with initial discussions centering around cooperation in the following areas: biochemical and epidemiological research related to asbestosis and silicosis; biochemical and epidemiological research related to heavy metals; development and validation of short-term test methods to detect and assess carcinogens, mutagens, and teratogens in the environment; and the application of standard toxicological test methods and the extrapolation of laboratory animal data to man.

US-Egypt Cooperation

Cooperation between American and Egyptian environmental health scientists is being carried out under the auspices of the U.S.-Egypt Joint Working Group on Health Cooperation (JWGHC). NIEHS has been assigned responsibility for the U.S. Subcommittee on Environmental and Occupational Health of the JWGHC. Efforts to date have consisted of a workshop held in Egypt in early 1980 to identify the biomedical impacts of technology transfer. During 1982, a series of training workshops were held in Egypt focusing on the following areas: pesticides, trace metals, radiation, environmental management, and mutagenesis. An information unit for environmental impact assessment is also being established. This project is directed to the needs expressed by the Egyptians for information in the areas of environmental and occupational health.

US-Japan Cooperation

Cooperation between American and Japanese scientists on environmental health problems takes place under two formal agreements: The U.S.-Japan Cooperative Medical Sciences Program and the Agreement on U.S.-Japan Cooperation in Research and Development in Science and Technology. Under the U.S.-Japan Cooperative Medical Sciences Program, American Environmental health scientists participate in the Panel on Environmental Mutagenesis and Carcinogenesis chaired by the Associate Director for Genetics, NIEHS. Joint areas of research focus on the detection of mutagenic and carcinogenic chemicals using both in vitro and in vivo test systems, and on monitoring human populations for evidence of exposure to mutagenic and carcinogenic chemicals. Joint research on carcinogens and mutagens in the diet and dietary tract have been particularly productive. Under the U.S.-Japan Agreement on Cooperation in Research and Development in Science and Technology, NIEHS participates in the toxicology program area in the counterpart working group on health. In September 1981, the Director, NIEHS, led

the U.S. counterpart working group on health at the first meeting of the Joint Committee for the U.S.-Japan Cooperation in Research and Development in Science and Technology, held in Tokyo, Japan. In the toxicology program area, discussions centered on cooperation in the following subjects: development and validation of short-term test methods to detect carcinogens and mutagens; development of methods to test volatile chemicals; development of methods to study mixtures of chemicals; studies on the chemical initiation and promotion of cancer; and the development of approaches to quantitative risk assessment.

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the *Medical Science and Public Health Cooperative Agreement*, scientists from both countries are conducting joint research on the effects of physical and chemical environmental agents on human health. 1982 was the tenth year of formal collaboration in environmental health research between the U.S. and U.S.S.R. The first year was concerned largely with establishing working relationships and agreeing on areas of joint study. Cooperative research efforts initiated in the second year involved exchange visits between scientists of both countries. Research results developed during the second and third years of collaboration were presented by American and Soviet scientists in the First Joint Symposium in Riga, Latvia, in December 1974. Scientific results from cooperative research during 1975 and 1976 were presented at the Second Joint Symposium, held in Marineland, Florida, in December 1976, and results of research conducted between 1977 and 1979 were presented at the Third Joint Symposium held in Suzdal, U.S.S.R., in October 1979. The proceedings of these symposia were published in both countries. During 1977, 1978, 1979, and 1981, major workshops were held on the following topics: developmental toxicology (Leningrad, November 1977); biological effects of metals (Cincinnati, February 1978); behavioral toxicology (Suzdal, November 1978); and biological effects of physical factors in the environment (Seattle, June 1979; and Kiev, May 1981). In May, 1982 a joint U.S.-U.S.S.R. workshop on "Nervous System Effects of Electromagnetic Waves" was held at NIEHS.

NIEHS also participates in the U.S.-U.S.S.R. Agreement on Cooperation in the Field of Environmental Protection which is administered for the United States by the Environmental Protection Agency. The Director, NIEHS, serves as DHHS representative to the Environmental Protection Agreement and co-chairman of the working group concerned with the biological and genetic effects of pollution. During 1980-1981, exchange visits under this Agreement were conducted in research areas concerned with the health effects of oil shale technology and the mutagenicity of environmental contaminants. In the area of mutagenesis, joint studies are being conducted to determine the significance of an increased mutation rate among congenitally malformed children in the U.S.S.R.

Cooperation with the World Health Organization (WHO)

NIEHS has been designated by WHO as a Collaborating Center for Environmental Health Effects since 1975. In 1979, WHO established the International Programme on Chemical Safety (IPCS), a cooperative undertaking involving WHO, the United Nations Environmental Programme, the International Labor Organization, and their member states. In October 1980, a cooperative agreement was signed between NIEHS and WHO, and NIEHS assumed the function of lead institution within the

IPCS for such activities as international evaluations of biological effects of chemicals and health hazard assessments, and review and/or validation of methods for testing of mutagenicity, carcinogenicity, neurobehavioral toxicity, and toxicity to reproductive function. In September 1981 the Agreement was extended for another year. In order to assist NIEHS participation in the IPCS, a WHO Inter-regional Research Unit was established at NIEHS in 1981.

The objectives of the IPCS are: 1) to encourage international cooperation in the evaluations of the effects of chemicals on human health and on the quality of the environment; 2) to coordinate chemical testing and toxicological research to eliminate unnecessary duplication of effort; 3) to develop international protocols for laboratory testing, epidemiological studies, and risk assessment; 4) to develop international guidelines and exposure limits for chemicals in air, water, and food and limits for hazardous chemicals in workplaces; 5) to develop response mechanisms for coping with chemical emergencies which may be international in scope; and 6) to promote training and development of manpower in areas and specialities necessary for the achievement of program goals.

Interagency Coordination

A number of federal and state agencies are involved in collaborative efforts to establish integrated systems for gathering, evaluating, and disseminating information on the health and environmental effects of chemical substances. The Assistant to the Director for International Affairs represents NIEHS on the Toxicology Information Subcommittee (TIS) of the DHHS Committee to Coordinate Environmental and Related Programs. This committee identifies the needs and establishes the mechanisms for the collection, storage, and dissemination of toxicologic information within DHHS. She is also the NIEHS representative on the Interagency Toxic Substances Data Committee (ITSDC), committee formed to design and coordinate an effective system for the retrieval of information on chemical substances submitted to EPA under the Toxic Substances Control Act, and a member of the Chemical Substances Information Network Subcommittee of the ITSDC which oversees the integration of a wide variety of data bases into one information network.

The Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA), provides for several Federal organizations to participate in a coordinated response to hazardous substances released into the environment or in the cleanup of hazardous waste disposal sites. In order to provide for the effective coordination of the collection, development, and evaluation of the information necessary to determine the potential health hazards associated with such chemicals, the DHHS Committee to Coordinate Environmental and Related Programs (CCERP) has established a Hazardous Waste Information Evaluation Subcommittee (HWIES). The HWIES will evaluate information collected about chemicals relevant to CERCLA, and make recommendations via the parent Committee concerning the testing of chemicals by the National Toxicology Program, and record creation by the National Library of Medicine. The Assistant to the Director for International Affairs chairs this new subcommittee.

PROGRAM PLANNING AND EVALUATION

OFFICE OF PROGRAM PLANNING AND EVALUATION
Summary Statement

During the past year the Office of Program Planning and Evaluation (OPPE) continued a broad range of planning, evaluation, program analysis, legislative support and related activities. The most significant of these are summarized below. As in previous reports, these activities are summarized under two broad categories:

1. Areas of substantive program activity.

2. Areas of functional activity.

For the convenience of those who might wish to learn more about particular OPPE activities the name of the staff member who worked on each is given.

Substantive Program Activities

Toxic Chemical Waste Dumps (Superfund) (Mr. Kingman, Ms. Hoffman)

The past year brought PHS implementation of its responsibilities under Superfund. The Centers for Disease Control (CDC) serves as the PHS focus for HHS responsibilities under the Act. OPPE carried out a variety of activities in support of both NTP and CCERP Superfund related activities, and in addition served as NTP liaison with CDC on Superfund issues. OPPE in collaboration with NTP staff, developed 1982, 1983, and 1984 NTP budget proposals for Superfund activities. Also, jointly with NLM, CDC and NIEHS staff, OPPE developed a proposal for a subcommittee of CCERP to provide review of the adequacy of the existing data base on the chemicals found in dumps, and where appropriate to propose that NTP test them. This interagency subcommittee is being chaired by the Special Assistant to the Director for International Affairs.

Health Promotion and Disease Prevention (Ms. Hudson)

In continuation of the Department's activities in prevention, and in response to the Secretary's interest in this area, there has been increased emphasis on prevention. The NIEHS Deputy Director is serving as Institute staff representative for prevention; and OPPE provides staff support to him and carries out other prevention activities. To date, this has involved preparing a summary of what NIEHS planned to emphasize with regard to disease prevention for inclusion in its opening statement for the Appropriations hearings; developing the FY 1983 NIEHS Disease Prevention Research Plans for the NIH Director's Fall Planning/Appropriation Briefing Session; developing the NIEHS Disease Prevention Research Plans for FY 1983-84 for the NIEHS FY 1983 Research Plan and the NIH Research Plan; and reviewing and preparing comments on planning documents for the NIH prevention initiatives effort.

Throughout the past year OPPE has been called upon to participate in and contribute to other Assistant Secretary for Health (ASH) activities related to its efforts to develop a DHHS-wide program in health promotion and disease prevention. This has required continuing input for and review of ASH-developed documents. For example, OPPE provided information on the NTP prevention efforts, prepared a description of NIEHS activities in prevention, and updated

FY 1980, 1981, and 1982 budget figures for prevention documents published by the Department (i.e., Prevention '80 and Prevention '81). OPPE also coordinated preparation of comments by toxic agent and radiation control work groups members on other implementation plans prepared for submission to ASH.

National Toxicology Program (NTP) (Ms. Hudson, Mr. Kingman)

As part of its continuing staff support to the Director, NTP, OPPE carried out a variety of activities. Among these were: Development of the NTP Program Performance Summary, which contained information on the program's background, including legislative history; program objectives and activities; evidence of program effectiveness; major issues confronting the program; and barriers that might affect program performance. This summary, which was developed to provide a synthesis of legislative, budget, planning, and evaluation perspectives on a high-interest topic for prompt response to Congressional, White House, OMB, and Departmental requests, was included in the FY 1982 Research Plan. OPPE also prepared a summary of NTP Accomplishments in FY 1981 and NTP Plans for FY 1983 and 1984 for the NIH Director's Planning/ Appropriation Briefing Session in the Fall, as well as for the NIEHS Director's briefing prior to his program review session at NIH. This summary was developed to provide the information and justification needed so that the NIH Director could establish tentative budget ceilings for NIH programs. It later was used to brief the Directors of the NIEHS-supported Environmental Health Sciences Centers at their February meeting.

Intergovernmental Relations (Ms. Hoffman)

While OPPE continues its interest in intergovernmental relations as they relate to environmental health problems, other more urgent staff assignments precluded much activity in this area. However, OPPE is participating on a committee of the Triangle J Council of Governments (a Research Triangle area intergovernmental body) which is studying hazardous waste accidents in this region.

Functional Activities

Program Planning Activities (Ms. Hoffman)

OPPE continues to be involved in a broad range of planning activities. Program review sessions with Dr. Rall and the OD staff, which are now held twice a year, are coordinated by OPPE. These reviews serve a number of functions and they prepare the Director for House and Senate Appropriations hearings, as well as for his briefing of the Director, NIH. They also serve as the basis for development of NIEHS' long term Research Plan and the budget justification. In carrying out the program reviews and in writing the Research Plan, OPPE works closely with Institute program and budget staff. OPPE is also serving on an Intra-NIH advisory group planning and overseeing a study of NIH planning processes.

Program Evaluation Activities (Ms. Hudson)

OPPE continues its involvement in a broad array of program evaluation activities. Key among them is the preparation of the Institute's Program Evaluation Plan. In addition, NIEHS is completing an evaluation of the NIEHS Environmental Toxicology Training Program, which has been funded with set-aside funds.

Objectives of the study are to analyze the environmental toxicologist supply and demand; project future needs; evaluate types of training needed for jobs in industry, academia, and government; and assess how the NIEHS training program impacts on institutional training. For the coming year OPPE has proposed an evaluation of the NIEHS epidemiology training programs. In addition to these activities, OPPE has been involved in a variety of long-term, continuing and one-time evaluation efforts.

During FY 1982, OPPE served as the NIEHS representative on the NIH Subcommittee to Integrate Evaluation Activities into the NIH Director's Review Session; this group recommended changes designed to integrate both planning and evaluation issues in the Director's review sessions with Institute Directors.

OPPE also has served on the NIH Evaluation Oversight Committee, the central NIH steering committee for evaluation, which has dealt with issues relating to reporting of evaluation activities and subsequent report use in research and evaluation plans.

Another project undertaken by OPPE was to coordinate preparation of material on the Environmental Mutagen Information Center (EMIC) and the Environmental Teratology Information Center (ETIC) for ASH's FY 1984 planning process. ASH established a working group to produce findings and recommendations in five areas of PHS policy; one group was charged with defining current relationships and interactions between PHS agencies and the private sector in the collection, analysis, and distribution of health data, with particular emphasis on methods of financing these activities. OPPE was asked to identify and describe agency-directed activities that provide information to the private sector (as well as the public sector) and private data sources used by the agency.

Legislative Analysis (Ms. Hudson, Mrs. Stopinski, Ms. Hoffman, Mr. Kingman)

The resignation of Mrs. Stopinski during the year caused a reduction in the ongoing OPPE legislative tracking and monitoring activities. However, OPPE staff did carry out a variety of legislative support projects. Among these were: In collaboration with Institute scientific staff preparing Institute comments on draft animal welfare legislation, assembling and preparing briefing material for House and Senate hearings on Superfund implementation and Love Canal, and preparing testimony and briefing material for a House hearing on Chemical Hazards to Human Reproduction.

Program Analysis (Mr. Kingman, Ms. Hoffman, Ms. Hudson)

Throughout the year OPPE was called upon to carry out a variety of ad hoc assignments. Among these were:

- A survey of research relevant to learning disabilities.
- A survey of information on use of animals at NIEHS.
- Surveys of NIEHS activities related to:
  --aging
  --ocean pollution
  --long-term care
  --orphan drugs

- A review of formal NIEHS interagency collaborative activities.

- A report to the Chairman of the House Committee on Appropriations on the toxicity of malathion (prepared in collaboration with the Office of Health Hazard Assessment).

In addition, during the summer, OPPE assumed responsibility for preparation of a proposal for an Institute-wide program information system. This proposal is being prepared in cooperation with Extramural Progam staff, with the collaboration and support of the Computer Technology Branch, BRAP. It is planned to have an action memorandum for the Director's decision by the end of September.

Administrative Activities

NIH is in the process of developing factor evaluation guidelines for the program analyst series. Eight program analysts in various Institutes and offices were asked to participate in a test application of the draft guidelines to their positions. Ms. Hoffman's position is part of this test. OPPE evaluated her position using the proposed guidelines. The personnel office conducted the same evaluation as well as re-evaluated the position under the existing system. The guidelines will be modified based on the results of this test, and following OD approval a workshop will be set up at NIH to instruct staff in their application.

This year brought significant staff turnover. Ms. Arner, Secretary, accepted another position in the Institute, Ms. Wilkins, Clerk-Typist, and Ms. Stopinski, Legislative Analyst, resigned. Of these positions only the Secretarial position has been filled, although, an acceptance has been received by the new clerk-typist, and an offer will soon be made to an individual for the legislative analyst position.

The loss of these individuals came at particularly difficult times. With regard to clerical support, OPPE was able to function at a high level of productivity because of the excellent support provided by Ms. Susan Taylor, a student at Appalachian State University, who joined the staff for the summer and quickly learned OPPE procedures and office equipment, and became fully productive in a short time. She will be sorely missed, and OPPE hopes she will be able to return in the Summer of 1983.

In addition, OPPE was able to take on a number of long-delayed tasks thanks to the presence of Ms. Carolyn Cantlay and Mr. Thomas Foard, graduate students. They provided fresh approaches and much needed hands and minds during a busy summer.

In terms of long-term benfits to OPPE and to the Institute, it is likely that the introduction of the word processor will prove to be the major improvement of the last decade. However, it is becoming apparent that OPPE is generating an increasing number of documents, and that over the long-term it is unrealistic to expect only two clerical staff to prepare them.

FACILITIES ENGINEERING

OFFICE OF FACILITIES ENGINEERING
Summary Statement

The Office of Facilities Engineering plans, directs, supervises and coordinates all facilities engineering activities including, but not limited to, engineering design, inspection, construction, master planning, operation of utility plants and systems, maintenance and repair of all real property (buildings, grounds, surfaced areas, utility plants and systems), maintenance of facility operations equipment and vehicles, fire prevention and protection, custodial, refuse collection and disposal, supply and storage of construction and operations materials, and other miscellaneous facilities engineering services and operations.

Office of Facilities Engineering personnel make environmental assessments and prepare impact statements relating to the preservation, protection and enhancement of the environment. At the direction of the Director, NIEHS, the Office personnel perform non-facility engineering mission support work including but not limited to security, graphics, photography, and the design, fabrication, alteration and repair of intramural scientific instrumentation.

Facilities: The Office of Facilities Engineering is responsible for the entire NIEHS facility currently comprised of 192,671 square feet of leased space and 452,273 square feet in government-owned facilities on the South Campus site. The North Campus site provides 154,000 square feet in a complex of 19 building, with a staff housing capability in excess of 500 permanent full-time employees.

The new NIEHS laboratory, administrative and support facilities under construction are on a 509-acre tract of land in the Research Triangle Park that is in close proximity to the North Campus facilities. The new facilities, designed to NIEHS requirements, were funded by a $67 million Congressional appropriation. A construction management firm was selected in December 1976; construction contracts were subsequently awarded and construction began in April 1977. The Shops Building, Warehouse, Power Plant and Administrative Modules A and B of Building 101 have been completed, accepted by NIEHS and occupied. Construction delays have resulted in a revised completion date of late 1982 for the laboratory modules. Upon occupancy of the South Campus facilities, NIEHS will continue to occupy the North Campus facilities, while "off site" leased facilities will be relinquished.

The table below outlines the function and amount of space in both the current leased and new Government owned facilities. Staffing capability is projected at approximately 800 for the new facility and 400 in the existing quarters.

Program Facility	Current Leased Facilities (gross sq.ft.)	South Campus Facility (gross sq.ft.)
Laboratories	52,883	135,340
Animal	15,356	86,560
Biostatistical Labs		23,460
Direct Lab Support	19,236	39,780
Office	35,671	28,480
Conference Facilities & Public Space	2,712	7,930
Cafeteria	2,111	12,070
General Support	6,725	
Support Services Plants	19,580	118,000
SUBTOTAL	154,274	451,620
Off-Site		
Trailer City	13,248	
East Campus	11,025	
Progress Center	5,130	
Northrop:		
Offices	3,824	
Laboratories	6,722	
Direct Lab Support	495	
Warehouse:		
Old Raleigh Road	15,500	
Asbestos Storage	6,166	
Neptune Storage	5,700	
Duke Animal Colony Bldg	6,500	
SUBTOTAL	74,310	
TOTAL	228,584	

Office Functional Units: The Office of Facilities Engineering is divided into five functional units in addition to the Office of the Chief. The Resource Management Section is the coordination point for all Office of Facilities Engineering service requests providing planning/estimating, maintenance scheduling, material expediting, shops materials and parts storage and disbursement services to OFE. The Resource Management Unit maintains all work order, contract and manpower management records. The Engineering Design Section provides the architectural and engineering support required for planning new NIEHS facilities, improvements, major repairs, consultation, liaison and review functions for projects contracted and administered by DES, NIH. The group also provides architectural and engineering support required for the administration and inspection of NIEHS construction provided under direct contract. This includes reviewing shop drawings and coordination input into the permanent site (South Campus) construction.

The Facilities Operations Unit oversees operations and maintenance of the power plant and building mechanical systems on the NIEHS South Campus. The power plant houses two 40-million BTUH oil, coal, and gas-fired boilers and two 2,500-ton chillers. These systems are in continuous operation and deliver the environmental control (heating, cooling and humidity) to all the facilities on the permanent site including the main NIEHS Administrative Laboratory Building (101). The Facilities Maintenance and Repair Section provides shop services to

the Institute. This includes 3 units: electrical, carpentry, and mechanical. Together these units respond to emergency repair calls, make slight structural modifications and otherwise perform maintenance, major equipment preventive maintenance and repair. Operations of leased facilities are managed by this section in concert with the Resource Management Section.

The Instrumentation and Arts Section provides arts, graphics and photography services to Institute personnel as adjuncts to publications of papers, conferences, seminars, and scientific exhibits. Additionally, the section provides instrumentation fabrication services; both from a machine and electronic view. The section also supports repair and maintenance.

Goals and Accomplishments:

In fiscal year 1982, the Office of Facilities Engineering has continued the process of reorganization and growth initiated in 1981 in order to meet its expanded mission in the most efficient manner possible. The Office of Facilities Engineering was reorganized into five sections and an OFE staffing plan was developed to utilize personnel in the most appropriate manner. A vacancy occurred in the Branch Chief position in June of 1981 and was not filled until October 1981. Normal staff turnover presented problems for the OFE because of the DHHS wide freeze on employment. Despite the time consuming requirements for requesting exemptions to the freeze, several key management positions were filled. Recruitment activities continue in order to fill the twenty vacant positions in the OFE.

The Engineering Design Section devoted a large effort toward the design of required North Campus renovations and modifications, and completions to the laboratories under construction in Modules C, D, and E of Building 101 on the South Campus. The renovations on the North Campus are required to up-grade deteriorating buildings and systems and to prepare for further back-filling by NIEHS personnel. The modifications and completions on the South Campus will be confined to those changes which are an absolute prerequisite to the functional occupancy of a laboratory or to provide services for the installation of special bench/casework or free-standing equipment on the bare wall present in most C, D, and E laboratory modules.

Office of Facilities Engineering management personnel continued to work closely with DHHS, ROFEC staff to reach an orderly and timely completion of the NIEHS South Campus facility. The Facilities Operations Unit and Facilities Maintenance and Repair Section are developing an overall strategy for the operation and maintenance of the South Campus facility and integrating it with the on-going activities of the North Campus.

The OFE concentrated on planning for the shakedown period in the new building with OFE craftsmen and operators attending classes offered by the manufacturers to learn the new systems which had been installed in the building. These classes were especially timely in light of OFE's goal of improved response time to work order requests coming into all sections. Management personnel in OFE strove to reiterate, in FY82, the need for OFE to be more responsive and supportive of the bench scientist community at NIEHS. Turnaround time was visibly improved in all areas, especially in art and photography. Additionally, OFE began implementation

of preventive maintenance support to the scientific community at the Institute by consolidating all service contract requests on distributed computer equipment. This should result in more funds available for pure research.

Future Branch Objectives:

During the next fiscal year, OFE plans to concentrate on three new areas in addition to continuing its efforts to see certain NIEHS laboratories relocated into the permanent facility on South Campus and renovations begin on North Campus. These areas of interest are: a) energy conservation, b) phased implementation of a preventive maintenance program for both building service and biomedical laboratory equipment and c) establishment of internship programs in the Instrumentation and Arts Section. The full occupation of the permanent facility on South Campus will enable OFE to make use of a computer system which will aid in managing energy resources. OFE's goal during the next year is to gain experience and collect and analyze data so that during ensuing years good energy conservation can be exercised. The move into the South Campus facility will also give OFE an opportunity to identify much of the biomedical laboratory equipment and place it on a preventive maintenance schedule. Establishment of internships in the electronics shop and art unit, we hope, will give OFE additional "hands" to support the increased requests for services generated by the investigators. We anticipate that with additional contracting out, and an internship program, more timely responses can be made.

HEALTH AND SAFETY OFFICE

HEALTH AND SAFETY OFFICE
Summary Statement

The health and safety program has broad responsibility for chemical and radiation safety, physical safety, fire protection, emergency preparedness, environmental protection and employee health surveillance. In FY82 these various programs were organized into the Health and Safety Office and a manager with overall responsibility recruited. Additional technical and clerical staff were added to this program to increase effectiveness and responsiveness to Institute needs.

The primary emphasis of the Health and Safety Office is prevention of exposures through utilization of containment equipment, following appropriate work practices and procedures and use of personal protective equipment. Prior to beginning studies, investigators prepare hazardous agent safety protocols which are reviewed by the Health and Safety Office as well as other selected Institute personnel. This review serves to identify and correct potential problems as well as to alert the safety program of needed monitoring studies. During FY82, the protocol system was improved and streamlined by merging the radiation safety and chemical safety protocol systems into one uniform protocol for hazardous materials. A computer system for maintaining records of active protocols also was developed to aid other investigators in preparing protocols for similar research and to assist the program in responsing to emergencies such as chemical spills.

Institute programs promoting safe use of hazardous chemical agents remain high priority. Routine duties include quarterly surveys of Institute facilities, exposure monitoring studies for selected work place contaminants and quarterly evaluations of laboratory hood and containment equipment performance. The Institute's chemical exposure monitoring program was much improved by establishment of an industrial hygiene laboratory in FY82. Routine and special exposure monitoring studies for noise, organic solvents, formaldehyde, anesthetic agents, asbestos, airborne particulates in animal facilities and selected chlorinated compounds were conducted. A special study was undertaken to evaluate performance of free standing laboratory hoods used at NIEHS for control of organic solvent vapors. Pilot studies also were conducted evaluating routine cage washing as a means of removing hazardous chemical and radioactive compounds.

The Institute's use of radioisotopes and radiation sources continues to increase. Routine duties of the radiation protection program include monthly laboratory surveys, surveys of sealed sources, checking for contamination in cases of suspected spills, receiving and surveying incoming isotopes, calibration of radiation detection instruments, disposal of radioactive wastes, bioassay procedures, monitoring of personal exposures and keeping an inventory of all radioisotopes at the Institute. The Institute's 1982 NRC inspection found no violations. Special studies conducted during FY82 included ambient monitoring and stack monitoring during incineration of liquid scintillation vials.

Disposal of hazardous materials generated as a result of Institute research activities received much attention during FY82. The Institute's overall hazardous waste management program was reviewed and areas of needed improvements identified. FY82 improvements included design of a packing and temporary storage facility meeting RCRA requirements, development of a contract for waste

pick-up and processing, updating RCRA required training and contingency plans and improvements in waste processing and disposal records. NIEHS currently disposes of most hazardous chemical wastes by burial in EPA approved landfills. Alternative methods such as incineration were investigated during FY82. Plans are being developed to convert one of the Institute's pathological incinerators to a high temperature chemical incinerator.

The Health and Safety Office manages the Institute's occupational medicine program. NIEHS currently participates in the Federal Employee Occupational Health Program for basic medical services and contracts with the Duke University, Occupational Health Service for special periodic surveillance examinations tailored to potential exposures of the individual. During FY82, these programs were reviewed and a contract scope of work developed for the NIEHS Occupational Medicine Clinic planned for the NIEHS permanent facilities. Plans are also underway for the Health and Safety Office to assume responsibility for managing the Institute's workmen's compensation program.

Safety and health training is an important component of the Institute's safety program. NIEHS policy requires that all new employees attend a course in General Laboratory Safety conducted by the Health and Safety Office. Personnel using radioisotopes are also required to attend the Institute's course Introduction to Radiation Safety. During FY82 the Health and Safety Office conducted four sessions of the General Laboratory Safety and the Radiation Safety courses.

Emergency preparedness received considerable attention during FY82. Occupant Emergency Plans were developed for all NIEHS facilities and rental areas. The Institute's Emergency Response Team as well as other Institute personnel received training in CPR and Treatment of Medical Emergencies. During FY82, four training sessions concerning selection and use of fire extinguishers were conducted.

LIBRARY

LIBRARY AND INFORMATION SERVICES OFFICE
Summary Statement

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include manual and computerized literature searching of more than 200 bibliographic data bases, maintenance of a collection of some 681 periodical titles and 8,500 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 1,520 new books for the Library and the laboratories, publication of a monthly newsletter, and compilation of the annual bibliography of publications by NIEHS personnel.

Reference/Literature Searching: The Library maintains one of the most up-to-date computerized literature searching capabilities in the world, with access to more than 200 data bases covering subjects from toxicology through business administration. During FY 82, Library personnel performed searches on 1,000 topics, usually using several data bases per question to ensure complete coverage. The most heavily used data bases continued to be TOXLINE, MEDLINE, Toxicology Data Bank, Biological Abstracts, and Chemical Abstracts. The Library added the Chemical Abstracts Service Online, a data base designed for chemical structure searches, and the Library became a test center for CSIN, the Chemical Substances Information Network. Also, the Library became the NIEHS focal point for the Nucleic Acids and Protein Sequence retrieval system.

Journal Collection: The journal literature continues to be the primary means of disseminating scientific information. Consequently, the Library emphasizes its journal collection in order to cover as many of the diverse subject areas as possible which fall under "environmental health."

The Library subscribed to several new serials during FY 82, bringing the total to 681. In addition, the Library ordered about 277 subscriptions for the various laboratories. To improve handling subscription orders, procurement authority for NIEHS was trasferred from NIH to the Library. The Library continued the policy of selectively binding journals or replacing them with microfilm to save space. Some older volumes were put in storage in a trailer annex. The collection now includes 10,500 journal volumes and 1,300 microfilm reels.

The Library, working with the EPA Library, produced a computer-generated journal holdings list updated for 1982.

Book Collection: Continuing the development of the book collection, the Library ordered 1,520 books in FY 82, a 25% reduction over FY 81, reflecting budget cuts and the substitution of subscription orders for a large number of books published in series form. Of the total number of books ordered, 40% were purchased for the Library and 60% for the Laboratories. The computerized on-order file continued to be crucial.

Responsibility for ordering government reports from the National Technical Information Service and the Government Printing Office was transferred from the Procurement Office to the Library during FY 82.

Through the Federal Library Committee, the Library continued using the automated

cataloging system, OCLC, a computerized union catalog of books held by more than 3,500 libraries nation-wide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for new books which already have cataloging data on OCLC. At a push of the button the cataloger can register a new book on the system. Continuing a joint project with EPA, the NIEHS Library processed the OCLC data into computer printouts which take the place of the card catalog. This computer-generated catalog contains title, author, and subject indexes for the entire NIEHS book collection. In the future, this system will present the capability of maintaining an online catalog for looking up books via computer terminals in the Library and in the laboratories.

Interlibrary Loan: There was an 18% decrease in the number of photocopy and book requests reducing the total to 12,975 in FY 82. Fortunately, 49% were filled from the Library collection and only 51% had to be sent to other libraries in and outside the U.S. The overall decrease in number of requests is a reflection of the availability of materials in the much-improved collection.

The OCLC computerized catalog, mentioned above, also proved useful for verifying titles for interlibrary loan and for locating libraries from which to borrow books. The interlibrary loan subsystem was used for borrowing books from libraries throughout the U.S. A side-effect was that more libraries were able to use the NIEHS collection as well.

Institute Manuscripts and Bibliography: The Library continued to maintain the NIEHS archives of manuscripts submitted for publication. Lists of new ones were published in the monthly newsletter. The Library published the 1981 NIEHS Bibliography, a catalog of the papers published by Institute personnel since 1966. The Bibliography was distributed to NIEHS authors and to interested parties in other government agencies and in industry.

Planning: Planning for library and information services to meet the needs of an expanded organization and new facility continued in FY 82. With the realization that the library planned for the South Campus would not be built in the near future, attention turned to reorganizing the space in Building 18. A major rearrangement of the bookshelves was made, some new shelves and study carrels were added, and some older journal volumes were moved to storage. However, there is only enough space left for one more year of acquisitions. If the Library staff can be relocated in offices elsewhere in the building, additional shelving can be built to satisfy the short-term needs.

The Library Director began action on the procurement of a computer system to automate the catalog and circulation functions. It is planned to be compatible with the system selected by the NIH Library.

A new reference librarian was hired to do in-depth library research for Institute scientists. The librarian also will promote library services and conduct seminars.

Close contact with various library and information organizations was maintained in FY 82. The Library Director took office as Chairman of the Special Libraries Association's Environmental Information Division. He was appointed to the N.C. Advisory Council for the Library Services and Construction Act, the body with oversight responsibility for the distribution of Federal funds to libraries. The Library hosted the N.C. Online Users Group workshop on in-house data bases.

INTRAMURAL RESEARCH PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR

LABORATORY OF ANIMAL GENETICS

LABORATORY OF ANIMAL GENETICS
Summary Statement

Research done by members of the Laboratory of Animal Genetics is designed to produce information about the nature and function of genes in eukaryotic organisms. This information is necessary to gain an understanding of the effects of environmental mutagens and carcinogens on living systems.

Eukaryotic Gene Structure and Regulation: Techniques for isolating, purifying, and characterizing DNA sequences are being used to examine the organization and regulation of genes in Drosophila. Two loci are being examined because they exhibit interesting structural and regulatory features. The white locus regulation is in part self-controlled and mediated through an interaction with a closely linked locus called zeste. The regulation is sensitive to spatial relationships of white genes within the nucleus such that chromosomal rearrangements that upset pairing of the locus perturb the function of the gene. This communication between alleles suggest a series of experiments that examine the role of RNAs in gene regulation.

The cut locus, also under investigation, presents an interesting organization that suggests that some regions of the gene are used to control gene function in tissue and stage specific fashion. The size of the gene, the pleiotropic nature of the alleles and the clustering of mutations with similar regulatory upsets within the gene make it a good model for examining the regulatory mechanisms of genes important in development.

A very important discovery is that in both the white and cut loci a majority of spontaneous mutations are associated with the insertion into the gene of DNA sequences of unknown origin and function. Many of the insertion mutations produce a partially functioning gene, as if the regulation of the locus had been modified while the protein-coding sequences remain intact. This suggests ways of studying the regulation of these genes to understand how mutagens can modify gene regulation as well as cause changes in the gene product.

The discovery of insertion/deletion changes in genes associated with spontaneous mutations has forced a revision of views about mutation processes and the factors that influence them. Of particular interest are the studies of hybrid dysgenesis, which is characterized by a 10-1000 fold increase in mutation rate when a family of insertion sequences in chromosomes is activated by placing chromosomes containing the insertions into a hybrid condition. The sensitivity of the insertion sequences to environmental factors is not known but such information is necessary if we are to understand fully all aspects of environmental mutagenesis. Important implications concerning the basis for spontaneous mutations in other species, including humans, also grow from these discoveries.

Genetic Control of Transcription: A major phase in gene expression is the transcription of DNA to form RNA, catalyzed by one of the RNA polymerases. RNA polymerase II, instrumental in the transcription of genes encoding proteins in higher organisms, is a heteromultimer composed of about 10 protein subunits. This enzyme complex and the genes that control it are under investigation by scientists at NIEHS in collaboration with a group at Duke University. The objectives are to understand the control of the transcription process, how the

multimeric protein interacts with DNA to recognize active genes from inactive ones, and how the complex interacts with other cellular factors influencing the specificity of transcription.

The approach to the analysis is through a genetical and biochemical characterization of genes encoding the enzyme complex. One locus, C4, that encodes one of the larger subunits, has been identified by virtue of a mutation that confers resistance to α-amanitin. Other genes are now being sought through genetic screens designed to pick out mutations that interact with the C4 mutations to enhance or suppress them.

A scheme patterned after the one used to clone the white locus has been used to identify clones of the C4 gene DNA sequences. Hybrid dysgenesis induced mutations at the C4 locus have been found to result from the insertion of a transposon of the P family into the C4 locus. By screening cloned DNA from such strains using labeled P transposon sequences as probes, the C4 locus sequences have been recognized and recovered in cloned form.

Chemical Analysis of Genes and Gene Products: This group is characterizing in detail the molecular structure of selected proteins and the genes that encode them. Objectives include understanding the secondary and higher orders of organization so important to the way proteins act in the cell. To fully appreciate the range of impacts that mutations have on cellular function and development, it becomes necessary to compare normal and mutant gene products in detail and to look at the evolutionary relationships among similar gene products in groups of related organisms.

The amino acid sequence of lactate dehydrogenase specific to the testis of the mouse (LDH-X) has been completely determined and 84% of the 330 residues of the rat LDH-X has also been sequenced. Ten percent of the 330 residues differ between the two LDH-X types, most of which can be accounted for by single nucleotide changes. Two-thirds of the differing residues are positioned on the surface of the molecules and may be involved in the antigenic determinants unique to the mouse and rat LDH-X isozymes.

The three dimensional structures of the molecules have been visualized by a computer program that can establish atomic relationships when supplied with the amino acid sequence. The LDH-X isozymes are being used by other groups at NIEHS as the basis for detecting mutations in single sperm cells. Mutations that modify the mouse LDH-X so that it is recognized by antibodies to the rat enzyme can be detected by fluorescence microscopy. The development of this mutation monitoring scheme depends directly on knowing the three dimensional structures of the isozymes and the types of mutational changes that can be detected antigenically.

Lactate dehydrogenase from mouse muscle (subunit A) and heart (subunit B) have also been studied, along with those from human heart, rabbit muscle, beef heart and horse muscle. The tryptic peptide maps and amino acid compositions have been determined, and it is evident that the muscle and heart subunits show a closer relationship to each other than to the testis specific form. The evolutionary relationships among the subunits and among species groups are emerging, helping us to understand the types and amounts of genetic diversity that exist and how that diversity has played a role in the evolutionary process.

The amino acid sequence data has provided a direct approach to the cloning of the LDH genes using recombinant DNA techniques. From the amino acid sequence, a short nucleotide sequence (approximately 14 nucleotides) that is not redundant and is unique to the gene encoding a specific LDH-X has been constructed. This synthetic polynucleotide is being used as a probe to identify the gene for LDH-X. Analysis of the DNA sequence encoding the protein and those flanking the structural gene is expected to yield important information about the gene's regulatory signals and allow evaluation of mutation events that perturb the regulatory mechanisms as well as those that modify the protein coding sequence itself.

Molecular Population Genetics: This group is studying the types and amounts of genetic variation at the DNA sequence level in natural populations of eukaryotic organisms. Such information is necessary to determine the effects of environmental mutagens on the genetic structure of populations. Most of the available information about the genetic architecture of populations has come from the examination of proteins. It is now clear that DNA sequences encoding proteins represent only a small fraction of the total information in chromosomes. It is, therefore, of great importance to extend our knowledge to include all types of genetic variation.

A survey has been made of the DNA composing the alcohol dehydrogenase locus (Adh) and adjoining regions in Drosophila melanogaster and related species. The patterns of restriction endonuclease sites in this region of the chromosomes were established and used to construct a molecular map of the gene and adjoining regions. The data collected show a large amount of variation consisting of insertions or deletions of DNA sequences flanking the structural gene for alcohol dehydrogenase. Since all of the Drosophila lines surveyed showed normal alcohol dehydrogenase activities, it is not clear what effect, if any, the insertion/deletion variations have on gene function.

A companion study focuses on the distribution of various transposable elements in a sample of chromosomes from natural populations. The interesting observation that different transposable element families tend to occur together at the same place in a chromosome opens questions that are important to an understanding of gene mutation processes concerning the nature of these elements, their origins and evolution.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60062-06 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Characterization of Isozymes and Mutant Enzymes in Mammals and Drosophila

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Other:	Farida S. Sharief	Biologist	LAG	NIEHS

COOPERATING UNITS (if any)
Departments of Biochemistry and Genetics, North Carolina State University, Raleigh, North Carolina

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The amino acid compositions of tryptic peptides of lactate dehydrogenase (LDH) isozymes from mouse muscle, mouse heart, human heart, beef heart, rabbit muscle and horse muscle, as well as α-glycerol phosphate dehydrogenase isozymes from Drosophila larva and adult, have been determined. The subunit A (muscle) and subunit B (heart) of mammalian LDH isozymes appear to be more closely related to each other than to subunit C (testis). The α-GDPH isozymes from Drosophila adult and larva appear to be coded by a single structural gene and different electrophoretic mobilities may be due to the post-translational modification. The structural characterization of the electrophoretic variants, F and UF isozymes of α-GDPH from Drosophila adults indicates the neutral amino acid substitutions as well as charge changes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The proteins were digested with trypsin, and tryptic peptides were analyzed on paper chromatography and electrophoresis at pH 4.4. The compositions of the eluded peptides were determined by automatic amino acid analyzer.

MAJOR FINDINGS AND PROPOSED COURSE: The peptide compositions of LDH subunits from mouse muscle and heart, rabbit muscle, horse muscle, bovine heart and human heart have been characterized. The partial sequence of mouse LDH-M has also been obtained. The primary structures of these mammalian LDH subunits obtained in this study are compared with the previously known sequences of somatic LDH-M and LDH-H isozymes from pig and chicken. About 50% of the 330 residues are identical among three LDH subunits M, H and X. The substrate and coenzyme binding sites are conserved in all of the known LDH sequences. However, the amino-terminal 20 residues appear to be extremely variable among three different subunits. The flexible loop region of testicular LDH-X sequences is markedly different from the corresponding segment of somatic LDH isozymes, Overall, the LDH-X (C) sequence failed to show a closer evolutionary relationship to the H(B) subunit than the M(A) subunit as previously proposed.

Compositional analysis of the soluble tryptic peptides representing about 70% of the 293 residues in the protein reveals a single peptide difference between the $GPDH^{F}$-1 (adult) and $GPDH^{F}$-3 (larval) isozymes. This peptide was shown to be the carboxyl terminus by sequence determination and by carboxypeptidase A digestion of the native protein. For $GPDH^{F}$-1, the sequence of the C-terminal tryptic peptide is Asn-His-Pro-Glu-His-Met-Gln-Asn-Leu-COOH, while that of $GPDH^{F}$-3 is Asn-His-Pro-Gly-His-Met-COOH. It is proposed that a proteolytic cleavage of the terminal three residues is involved in the post-translational control of GPDH isozyme expression. A comparison of compositions of soluble tryptic peptides representing about 50% of the 293 residues of $GPDH^{UF}$-1 with those of $GPDH^{F}$-1 reveals three different peptides. A neutral substitution of isoleucine for leucine was identified in a tripeptide (Ile, His, Lys) of $GPDH^{UF}$-1. Two different peptides (Phe, His, Arg for $GPDH^{F}$-1 and Met, Leu, Lys for $GPDH^{UF}$-1) were also found.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The structural characterization of various mammalian lactate dehydrogenase isozymes demonstrates the evolutionary relationship of LDH gene loci A, B and C. The molecular characterization of mutant isozymes of Drosophila α-GDPH elucidates the nature of genetic mutations and leads to the understanding of how genes in eukaryotes function.

PUBLICATIONS

Niesel, D.W., Pan, Y-C.E., Bewley, G.C., Armstrong, F.B. and Li, S.S-L.: Structural analysis of the soluble sn-glycerol 3-phosphate dehydrogenase isozymes and allelic variants of Drosophila melanogaster. J. Biol. Chem. 257: 979-983, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60098-03 LAG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Amino Acid Sequences and Antigenic Structure of Mammalian Lactate Dehydrogenase Isozymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Other:	Yu-Ching E. Pan	Visiting Associate	LAG	NIEHS
	Farida S. Sharief	Biologist	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lactate dehydrogenase isozymes from mouse testis and rat testis have been fragmented into small peptides by chemical and enzymatic cleavage. Most of these peptides have been separated and their amino acid sequences determined. One-hundred percent of mouse LDH-X and 84% of rat LDH-X (330 amino acids in each protein) have been sequenced. Immunological properties of LDH-X isozymes from mouse and rat have also been characterized.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purified lactate dehydrogenase proteins had been cleaved into small peptides by CNBr and trypsin. These peptides were separated by gel-filtration and ion-exchange chromatography. Amino acid sequences of the purified peptides have been determined by automatic protein/peptide sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: Both rat and mouse LDH-X isozymes have been shown to possess specific, as well as common, antigenic determinants by Ouchterloney double diffusion analysis and by enzyme immunoinactivation studies with rabbit antisera. The amino acid sequences of 100% of the 330 residues from mouse testicular LDH-X and 84% of rat LDH-X have been determined. Ten percent of the 330 residues are different between these two LDH-X sequences, and most of these differences can be explained by single nucleotide changes. Two-thirds of these different residues, which are on the surface of the LDH-X molecule, may be involved in the unique antigenic determinants of the rat and the mouse LDH-X isozymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The amino acid sequence differences among LDH-X isozymes will be correlated with the antigenic properties of mammalian LDH isozymes. The monospecific antibodies against testicular LDH-X isozymes will be used for monitoring of genetic mutations caused by environmental mutagens and carcinogens in human population. The chemical sequence of mouse LDH-X will also be correlated with X-ray diffraction data in order to understand the structure-function relationship of this enzyme.

PUBLICATIONS

Li, S.S.-L., Pan, Y.-C.E., Feldman, R.J., Fitch, W.M. and Sharief, F.S.: Structure and evolution of mammalian sperm-specific lactate dehydrogenase-X isozymes. Fed. Proc. 41: 1180, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60099-03 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Organization-Regulation of Mammalian LDH Genes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Other:	Kenneth S. Fong	Senior Staff Fellow	LAG	NIEHS
	Yu-Ching E. Pan	Visiting Associate	LAG	NIEHS
	Kunihisa Akai	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The in vitro translated LDH-X of mouse testis poly A-RNA was identified by specific immunoprecipitation using rabbit anti-LDH serum. Several mouse LDH-A cDNA clones have been isolated, and partial nucleotide sequence of recombinant plasmid pMLA73 has been determined.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The total RNA was extracted by phenol/chloroform procedure from the testes of mature mice (16 weeks old), and poly(A)-containing RNA was obtained by passing the total RNA over oligo(dT)-cellulose twice. The functional poly(A)-RNA was translated in cell-free protein-synthesizing systems from both rabbit reticulocyte and wheat germ lysates. The in vitro translated LDH-X was immunoprecipitated by anti-LDH-X serum followed by goat anti-rabbit IgG antibodies. Recombinant plasmids of mouse LDH-A cDNA were isolated by colony hybridization using the 500 bp probe of rat pRLD42.

MAJOR FINDINGS AND PROPOSED COURSE: The in vitro translation products, labeled with ^{35}S-methionine or 3H-leucine, appeared as many discrete bands on SDS-polyacrylamide gel. This pattern of radioactive protein bands differs from that translated from mouse liver poly(A)-RNA. A single band of radioactive protein corresponding to an apparent molecular weight of 35,000 was found by specific immunoprecipitation with rabbit antiserum to LDH-X isozyme. This band was not found in the cell-free translation products of mouse liver poly(A)-RNA immunoprecipitated with rabbit anti-LDH-X antiserum. This putative cell-free translated LDH-X polypeptide, which seems to be approximately 1% of total in vitro translated proteins, is being further characterized.

Several mouse LDH-A clones have been isolated, and partial nucleotide sequence of pMLA73 has been determined. This plasmid cDNA insert containing the coding sequence of approximately 400 bp and 3' untranslated region of about 500 bp.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In mammals and birds the LDH-X(C) isozyme is found only in testes and spermatozoa, whereas the LDH-M(A) and LDH-H(B) isozymes are present predominantly in skeletal muscle and in heart, respectively. It is of considerable interest to elucidate the genetic mechanism(s) underlying the tissue-specific expression of LDH subunits encoded by three different gene loci.

PUBLICATIONS

Okabe, M., Akai, K. and Li, S.S.-L.: Identification of lactate dehydrogenase-X translated in vitro from mouse testicular poly A-containing mRNA. Int. J. Biochem. 14: 371-375, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61005-03 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Analysis of RNA Polymerase II Function in Drosophila melanogaster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert A. Voelker	Research Geneticist	LAG	NIEHS
Other:	Shu-Mei S. Huang	Biological Laboratory Technician	LAG	NIEHS
	G. Bruce Wisely	Biological Laboratory Technician	LAG	NIEHS
	Henrik Gyurkovics	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

Dr. A. Greenleaf, Department of Biochemistry, Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to determine the structure:function relationships of the components of RNA polymerase II transcription complex in Drosophila melanogaster. RNA polymerase II is a heteromultimer consisting of approximately ten different subunits, each of which is presumably specified by a different locus. The number of associated transcription factors (which are not structurally a part of RNA polymerase II) is unknown, but evidence for their existence has been found in other systems. To date only the genetic locus which specifies α-amanitin-resistance to RNA polymerase II has been identified. That locus has been cloned out and its DNA is being analyzed to determine which subunit it encodes and the nature of the genetic control at the locus.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The basic approach of this study is to utilize genetic techniques of analysis to identify mutants of RNA polymerase II that can be subsequently analyzed at the biochemical and molecular levels. The one locus identified was discovered by screening for α-amanitin-resistance. The locus was mapped by standard recombinational and deletion mapping techniques. It was further characterized by induction and molecular characterization of additional mutants at the locus. Mutants at loci coding for other polymerase II subunits will be identified by mutagenesis of wild type flies and subsequent screening designed to detect one or more of the following: (1) modification of the α-amanitin-resistance allele; (2) modification of visible phenotypes known to be conditioned by the α-amanitin-resistance allele; (3) non-allelic suppression of temperature-sensitive lethality at the already-identified locus; (4) synthetic lethal interactions between newly induced mutants and specific alleles at the already-identified locus. Once new loci are identified by screens, they will be characterized by the genetic analytical techniques mentioned above.

MAJOR FINDINGS AND PROPOSED COURSE: A locus which confers α-amanitin resistance to RNA polymerase II has been identified. The locus is lethal-mutable. Different alleles at the locus affect male fertility and act as an enhancer of alleles at the other loci. To date, we have recovered at least 8 temperature-sensitive mutants. Additionally, a number of incompletely characterized mutants show promising results. Drosophila stocks (strains) that are essential to the recovery of suppressors are being constructed. We will use these specially synthesized strains to identify genes coding for other elements of RNA polymerase II structure and function. The C4 locus has been cloned out by identifying and recovering mutants at the locus which were caused by hybrid-dysgenesis-induced insertion of the P-element transposon. The probing of polyadenylated RNA by DNA clones from this region has identified several mRNA's which hybridize, suggesting that one of them may be the message which encodes the subunit which can be modified to confer α-amanitin resistance. Work is continuing to identify which of the subunits identifiable on SDS gels is produced by this gene.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: RNA polymerase II is an important enzyme in eukaryotic gene regulation and development. The details of the role of this enzyme are very poorly understood, and the prospects of a comprehensive genetic and biochemical analysis of such a complex enzyme with mammals are very bleak. Therefore we have chosen to approach the problem with Drosophila, a well-defined eukaryotic genetic system which allows a powerful combination of genetics and biochemistry. Moreover, it appears that the structure and function of Drosophila RNA polymerase II is very similar to that in humans and other mammals; thus, knowledge obtained in Drosophila can probably be transferred to and utilized in the human situation with relatively little modification.

Before we can understand the risks of environmental mutagens and carcinogens, we must know how they affect the fundamental processes of cell growth and development. The effort of this study is to determine the role of RNA polymerase II in normal cell function. When that knowledge is available, we can begin to assess how the various environmental insults impinge on normal cellular function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61011-03 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Regulation of Gene Function in Drosophila

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Burke H. Judd	Chief	LAG	NIEHS
Other:	Margaret W. Shen	Biologist	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a study of two closely related aspects of gene expression and interaction: allelic complementation and transvection. Interactions between alleles of some loci show that pairing or close association between homologous chromosomes plays a role in their function and regulation. The objectives are to discover how general this phenomenon is and what the molecular basis for the communication between alleles is.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The approach is through the genetic analysis of loci known to exhibit either allelic complementation or transvection or both. New alleles are induced by treating male Drosophila with x-rays or a chemical mutagen. Analysis of other complementation pattern is by standard methods for creating heterozygous conditions. Cytological analysis of polytene chromosomes is by phase microscopy of smears of salivary gland cells.

MAJOR FINDINGS AND PROPOSED COURSE: We have concentrated on two loci in Drosophila that show different aspects of allelic interaction. The white locus exhibits some unusual characteristics in its regulation when homologous X chromosomes are paired vs. unpaired. The locus is repressed by the zeste mutation if two w^+ loci are paired or closely associated but both w^+ alleles are active if they are unable to pair. We have determined that it is the proximal segment of the w^+ locus that is important in this communication between alleles. The most recent study involved the molecular characterization of a series of alleles that show mosaic function of the gene. These are being compared to derivatives that have lost the mosaic (autonomous) expression. The second locus under study is cut. Alleles at this locus show complementation between a group of alleles that modify the morphology of the wing and a group that modify the structure of the legs. Both groups fail to complement a cluster of lethal alleles that map at the proximal border of the locus. We are treating chromosomes carrying different ct alleles with x-rays and testing them for transvection effects. Several rearrangements have been recovered but none confer a transvection effect on the interaction of mutant alleles. We have discovered that there is an interesting interaction between the cut and Notch loci. Some N mutations fail to complement with cut locus deficiencies and some of the visible cut alleles. The use of Notch to distinguish different types of cut alleles should prove to be a useful tool in analysis of the regulation of the cut and Notch loci.

The proposed course for this study is to carry the analysis of the allelic interactions to the molecular level. Analysis of mutants that upset the allelic interaction will be done in terms of the molecular structures of the mutant gene compared to normal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding the mechanism of gene action and regulation in the development and function of eukaryotic organisms is central to solutions of problems concerning mutation by environmental agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61016-02 LAG

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Basis of PM Hybrid Dysgenesis in Drosophila melanogaster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Paul M. Bingham Staff Fellow LAG NIEHS

COOPERATING UNITS (if any)

M. G. Kidwell, Brown University, Providence, Rhode Island
G. M. Rubin, Carnegie Institution of Washington, Baltimore, Maryland

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular basis for the phenomenon known as hybrid dysgenesis in PM strains of Drosophila melanogaster has been determined. The mobilization of a transposable DNA sequence family present in P strains but not in M strains is responsible for the burst of spontaneous mutation seen in the offspring of P males crossed to M females.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: We are using both genetic techniques and the techniques of nucleic acid biochemistry.

MAJOR FINDINGS AND PROPOSED COURSE: We have shown to date that two of two white mutants arising in PM dysgenic hybrids result from the insertion of members of a single transposon family (as defined by the presence of extensive DNA sequence homology between the two elements). This transposon family is present (30 - 50 copies) in P strains of D. melanogaster and is missing entirely (less than one copy of 0.2 kb of homology) in M strains. We have further demonstrated that transposition of this transposon onto the X chromosome (from donor copies on the autosome) occurs very frequently (on the order of 5 copies per chromosome per generation) in PM dysgenic hybrids. We are currently characterizing other mutants arising in PM dysgenic hybrids (both at white and at other loci), the physical structure of the transposon and various other properties of the phenomenon.

In overview, our results to date strongly suggest that the dramatic stimulation of spontaneous mutation rate in PM dysgenic hybrids results from the mobilization (persuant upon outbreeding) of a particular transposon family. If this phenomenon is general, it may represent a (the) major source of spontaneous mutations in animal populations in nature, including human populations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phenomenon of hybrid dysgenesis may be responsible for much or most of the spontaneous mutations arising in natural populations of Drosophila melanogaster. Comparable phenomena may occur in other species, including humans, and the analysis of the phenomenon in Drosophila is expected to define the approaches for its analysis in other organisms.

PUBLICATIONS

Bingham, P.M., Kidwell, M.G., and Rubin, G.M.: The molecular basis of P-M hybrid dysgenesis: The role of the P element, a P-strain-specific transposon family. Cell, in press.

Rubin, G.M., Kidwell, M.G., and Bingham, P.M.: The molecular basis of P-M hybrid dysgenesis: The nature of induced mutations. Cell, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61018-02 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Population Genetic Variation in DNA Sequences

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Langley	Research Geneticist	LAG	NIEHS
Other:	Charles F. Aquadro	Staff Fellow	LAG	NIEHS
	Elizabeth A. Montgomery	Biological Laboratory Technician	LAG	NIEHS

COOPERATING UNITS (if any)
C. Laurie-Ahlberg, Associate Professor of Genetics, North Carolina State University, Raleigh, North Carolina

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Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Variation in the restriction map in the Adh region (alcohol dehydrogenase locus) of chromosome II of Drosophila melanogaster and some related species were examined. Insertion/deletion differences outside the transcriptional unit were common. The distribution within Drosophila melanogaster and among the species suggest that such variants are deterious mutants, although they do not have any obvious effect on Adh function. This initial observation is being repeated with a larger sample. It is also being extended to other loci. Gene activity of the various restriction map types is being measured to find evidence of the effect to the insertion/deletion variants.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Genetically isolated second chromosomes of Drosophila melanogaster and various isofemale lines of related species were reared and nuclear DNA isolated. This was digested with various restriction enzymes, electrophoresed, blotted (Southern) and probed with several cloned sequences from the Adh, Ddc and Lcp1 regions. Restriction maps were constructed and insertion/deletion sites cloned and analyzed.

MAJOR FINDINGS AND PROPOSED COURSE: Three restriction site polymorphisms and four insertion/deletion polymorphisms within the 12 kilobase region were found in natural population samples of Drosophila melanogaster (all flanking the Adh transcription unit). On a per nucleotide basis, there was more insertion/deletion than restriction site variability. Each variant was limited to one geographical locality suggesting limited spread perhaps due to deleterious effects. There is little insertion/deletion evolution among D. melanogaster and its closest relatives. This work confirms the results from investigation of many laboratory populations of Drosophila that insertion/deletion variability is common. It provides a foundation to the further investigation of these mutations and their consequences.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The assessment of risk to the human population from exposure to environmental mutagens depends on a solid understanding of population genetics. The potential significance of insertion/deletion variants in and outside transcriptional units is unclear but the question is approachable.

PUBLICATIONS

Langley, C.H., Montgomery, E.A. and Quattlebaum, W.F.: Restriction map variation in the Adh region of Drosophila. Proc. Natl. Acad. Sci. (USA), in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61019-02 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Collaborative Protein Sequencing

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Steven S.-L. Li	Research Geneticist	LAG NIEHS
Other:	Yu-Ching E. Pan	Visiting Associate	LAG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The complete primary structure of the 90 residues of a seminal vesicles secretory protein (SVS-IV) from rat has been sequenced. Sequence information of about 95% of the 186 residues from human dihydrofolate reductase has been obtained.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Protein sequencing: purified protein was fragmented into small peptides by chemical cleavage and enzyme digestion. Peptides were then purified by the combination of paper chromatography and electrophoresis. The amino acid compositions and sequences of purified peptides were determined by amino acid analyzer and automatic protein and peptide sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: The amino acid sequence of 90 residues of SVS-IV protein was determined and about 95% sequence information of human dihydrofolate reductase was obtained. The sequence information is being used to locate the cDNA and to study the structure of genomic DNA of these two proteins. The primary structure information is also helpful to understand the structure-function relationship of SVS-IV and dihydrofolate reductase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The primary structure information of protein is very important in elucidating the fundamental biological function. The collaborative research of protein sequencing will provide expertise so that fast and accurate information can be obtained for many projects currently carried out in the Institute.

PUBLICATIONS

Pan, Y-C.E., and Li, S.S.-L.: Structure of rat seminal vesicle secretory protein. Int. J. Peptide Protein Res., in press, 1982.

Pan, Y.-C.E., Domin, B.A., Li, S.S.-L. and Cheng, Y.-C.: Primary structure of dehydrogenase reductase from human methotrexate resistant KB/6b cell line. Fed. Proc. 41: 1180, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61021-01 LAG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Analysis of cut Locus of Drosophila melanogaster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph W. Jack	Staff Fellow	LAG	NIEHS
Other:	Angela E. Lane	Biological Laboratory Technician	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to determine how tissue specific regulation is accomplished for a particular gene in Drosophila melanogaster. This goal will be approached by studying the activity of the normally functioning gene and mutants of the gene that affect specific tissues. Cloned DNA segments will be used to study the structure of normal and mutant genes and the RNA products of these genes. I hope to understand how a newly recognized class of mutators, mobile DNA elements, disrupt the activity of the gene in question. These mobile elements are apparently present in many types of organisms, and Drosophila offers an opportunity to understand what effects the elements may have on a gene's activity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cloned cut locus DNA will be used to determine the DNA structure and RNA producing activity in wild type flies and flies with various cut locus mutations. In addition, new mutants will be induced using X-rays, transposable elements, and possibly other mutagens. Reversions of existing cut mutations will be sought and studied to determine what kinds of changes will relieve the mutational effects of the DNA insertion mutants.

MAJOR FINDINGS AND PROPOSED COURSE: In the past year, we have cloned a large portion of the cut locus DNA and have located the sites of a number of the mutants that affect specific tissues. Each mutant has proven to be a DNA insert of 5-10 kilobases into the cut locus DNA. A comparison of recombination distance between mutants to the distance on the DNA map reveals that the locus is large and is likely to be around 100 kilobases in length. We are now beginning work to determine what parts of the gene produce an RNA, the size of the RNA, and what tissues produce cut locus RNA products. The gene product is apparently a polyadenylated RNA and is thus likely to be a messenger. The structure of cut locus RNA products produced in cut mutants will be compared to the RNA's made by wild type cut alleles. We will also determine in which tissues the RNA production is affected by the mutants. We intend to clone DNA from mutant cut loci to learn more about the structure of the inserted DNA. We will also clone the remainder of the cut locus from wild type Drosophila clone libraries.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Transposable DNA elements are found in a number of widely different organisms. The elements are know to induce mutations, and some types induce tumors. This work is aimed at understanding how such elements can alter the normal activity and control of a gene.

Furthermore, the cut locus promises to be an excellent manipulable system to answer the question of how a gene's activity is controlled in a tissue specific manner.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61022-01 LAG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Population Genetics of Transposable Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Langley	Research Geneticist	LAG	NIEHS
Other:	John F.Y. Brookfield	Visiting Fellow	LAG	NIEHS
	Antony E. Shrimpton	Visiting Fellow	LAG	NIEHS
	Elizabeth A. Montgomery	Biological Laboratory Technician	LAG	NIEHS

COOPERATING UNITS (if any)

N. Kaplan, BRAP, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The distribution of transposable elements (copia, 412 and 297) were surveyed by in situ hybridization to 20 independent wild caught X chromosomes. The frequencies of various sites indicated that no site is commonly occupied. A theoretical model of the evolution of transposable elements in Mendelian populations was put forward. The analysis suggested that the distribution found in the X chromosomes is consistent with average number of transpositions per copy in the species as a whole being greater than 50.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: X chromosomes of Drosophila melanogaster were extracted and surveyed by in situ hybridization for cytogenetic location of the transposable elements: copia, 412 and 297.

MAJOR FINDINGS AND PROPOSED COURSE: No site was occupied in more than a quarter of the sampled chromosomes. This indicated that no sites are fixed in the species. There was a clear and interesting indication that elements from different transposable element families tend to occur together at the same site in the same chromosome. A population genetics model of the evolution of transposable elements in an outbreeding population was proposed and analyzed. The theory predicts distributions similar to those seen in the summary. In situations where the frequencies of occupied sites reach more intermediate frequencies, the theory affords the opportunity to statistically test the assumptions of the model. The proposed course is to characterize the distributions and structures of transposable elements in D. melanogaster and related species to gather a more general impression of this new class of organisms. Such analysis may lead to some understanding of the origins of transposable elements and their dynamics in the host populations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mounting evidence suggests that many "spontaneous" mutations are due to insertion of transposable elements. The insertion of transposable-element-like retrovirus sequences are implicated in oncogenesis. The understanding of the population biology of these chromosomal parasites will be essential in evaluating population risk to both mutagenesis and cancer.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY
Summary Statement

The scientific efforts of the Laboratory of Behavioral and Neurological Toxicology (LBNT) are directed toward the understanding of the behavioral and neurological effects produced by toxic substances. Special emphasis is placed upon the changes in behavior and neurologic function produced by long-term exposure to low levels of a wide variety of chemical and physical agents present in the environment and to exposure during the development of the nervous system.

The goals of the Laboratory are (1) to identify laboratory procedures useful in assessing the role of environmental factors in the development of behavioral and neurological abnormalities, (2) determine the mechanism(s) whereby environmental factors produce their behavioral and neurotoxic effects, and (3) identification of conditions which predispose individuals to the behavioral and neurotoxic effects of environmental factors.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving national and international programs and adjunct appointments. Training opportunities exist for graduate students at nearby universities, foreign and American postdoctoral scientists, and American scientists on sabbatical leave. Current expertise is in the areas of behavior, neurochemistry, and neurophysiology.

BEHAVIORAL TOXICOLOGY

The research of this group is devoted to assessing the effects of environmental factors on behavioral and neurological function and in determining conditions which predispose individuals to the behavioral and neurotoxic effects of these factors with increasing emphasis on assessing the consequences of exposure to environmental agents during the development of the nervous system.

Group scientists have developed a simple test battery for the neurobehavioral assessment of potential neurotoxins both in adult and young rodents. The tests include gross observations of sensory/motor functioning, hindlimb extensor response, forelimb and hindlimb grip strength, startle responsiveness, tremor, per formance on an inclined screen, visual placement, rectal temperature, spotaneous motor activity, and measures of acquisition and retention of a learned response. These tests have recently been standardized and validated using compounds with known neurotoxic effects.

Development of an analogous battery is in progress for use with Japanese quail. Tests of visual function, auditory function, pain sensitivity, and vestibular function are being developed as well as measures of spontaneous activity, neuromuscular function, and arousal/reactivity. The ultimate goal is to be able to make comparisons of neurobehavioral toxicity across a range of species. This is important in order to reduce the possibility of overlooking an effect due to a species idiosyncrasy and to enhance the extrapolation of results to humans.

Effects of acrylamide, triethyl tin, Kepone, and 2,4-dichlorophenoxyacetic acid have been studied in adult rats. Acrylamide is a known neurotoxin having effects generally most easily seen as peripheral neuropathy. This compound was studied in order to more clearly define the validity of the simple test battery. As expected, deficits in hindlimb function have been found with doses which do not affect forelimb strength further illustrating the usefulness of the two test procedures developed to measure these functions. In addition, the effect of acrylamide on motor responses under control of operant schedules was investigated. Acrylamide was also studied for its effects on peripheral sensation using an operant titration schedule. In this procedure, the rat "informs" the investigator of the point it perceives a shock to its tail by poking its nose in a tube containing a photoelectric cell detector. Other studies with acrylamide concerned its effects on food and water consumption, body weight, and spontaneous activity of rats.

Triethyl tin is being studied in an attempt to correlate the sensitivity of select functional tests with the pathology of tin toxicity and with changes in behavioral responses such as spontaneous motor activity and consummatory behaviors. Repeated administration of triethyl tin bromide (1, 2, and 3 mg/kg, orally) to adult male rats produced dose- and time-dependent reductions in body weights and food and water consumption. Tests showed decreases in fore- and hindlimb grip strength and startle responsiveness. Histologic examination immediately after the two week dosing period showed that in all dose groups there was intramyelinic edema of major central nervous system white matter tracts, the severity of which varied according to the dose.

In developing animals, triethyl tin given in a single dose on day 4, postnatally, produced long-term alterations in neurobehavioral functioning in the absence of statistically significant decreases in body weight. These effects could be categorized as increases in motor activity and altered reactivity (impaired active avoidance, elevated latencies to emerge into novel environment). Subsequent experiments found that neonatal exposure to triethyl tin resulted in an alteration in responsiveness to the stereotypic effects of apomorphine, a dopamine receptor agaonist. Postnatal exposure to triethyl tin was also found to alter the responsiveness of exposed animals in a two-choice visual discrimination task. Triethyl tin-exposed rats responding on the visual discrimination task showed a greater loss of stimulus control during reversal of the contingencies than controls. These experiments demonstrate that postnatal exposure to triethyl tin produces alterations in the ontogeny of basic neurobehavioral processes, including motor activity and startle responsiveness. Alterations in the ability to adapt to novel conditions (emergence) or learning (shuttle box, discrimination reversal) situations are apparent. Changes in postsynaptic dopamine function, but not affinity or density of dopamine receptors, seems to be affected by postnatal exposure to triethyl tin.

Previous research involving repeated exposure to chlordecone (Kepone) via the diet indicated that the adult rat displayed the essential features of neurotoxicity described for humans exposed to chlordecone (hyperexcitability, short-term memory loss, tremor). Subsequent research lead to the development of a technique to quantify the tremorgenic effects of chlordecone. Neuropharmacological studies revealed that chlordecone-induced tremor could be blocked or attenuated by trihexyphenidyl (a muscarinic receptor blocker), mecamylamine (a nicotinic antagonist), pizotifen (a serotonergic receptor blocker), propranol (a β-adrenergic

blocker), chlordiazepoxide (an anxiolytic), and muscimol (a GABA agonist). Yombine, a central α_2 adrenergic antagonist, appeared to exacerbate the tremor produced by chlordecone. Although chlordecone potentiated the lethal effects of strychnine, subconvulsive doses of strychnine did not alter the dose response curve to chlordecone. Haloperidol (a dopamine antagonist), quipazine (a 5-HT agonist), and clonidine (an α-agonist) had little or no effect on chlordecone-induced tremor. These experiments suggest that chlordecone acts at spinal and supraspinal sites. The pharmacological profile exhibited by chlordecone is consistent with the involvement of a caudate-cerebellar pathway. As in adults, administration of chlordecone to neonates produces a measurable tremor. One important difference between adults and neonates concerns their relative sensitivity to chlordecone. Neonates given chlordecone do not display tremors until at least 3 days postdose; adults begin to tremor as early as 1 hr postdosing. These data suggest that there is a neuronal pathway that mediates chlordecone-induced tremor and that this pathway does not appear ontogenically until at least day 7 postnatally. Subsequent experiments on the ontogeny of tremor using chlordecone and other known tremorgens are underway. One of the prominent effects of chlordecone following neonatal exposure appears to be some alteration in emotionality or reactivity. Chlordecone-exposed animals are hyperresponsive to a novel auditory startle stimulus; chlordecone appears not to affect the rate to habituation to repeated presentations of an auditory stimulus.

2,4-Dichlorophenoxyacetic acid (2,4-D) is being studied for its neurobehavioral toxicity in rats. This compound is a widely used herbicide and a component of Agent Orange. There have been scattered reports of delayed neuropathy following exposure to this agent. The consequences of exposure to 2,4-D on neurobehavioral functioning have not been studied systematically in animal models. 2,4-D was found to increase fore- and hindlimb grip strength in rats. This effect lasted for up to 6 weeks postdosing, depending on the dose regimen. However, the effect is not characteristic of agents that produce peripheral neuropathies. Such agents produce neuromuscular weakness in screening tests; 2,4-D had the opposite effect. Nonetheless, the fact that a persistent effect was observed demonstrates that effects of prolonged exposure to 2,4-D on neurobehavioral functioning needs to be systematically investigated.

NEUROCHEMISTRY

The primary goal of the Neurochemistry Workgroup is concerned with determining the biochemical bases for the effects of environmental agents on behavior and neurological function.

A series of high affinity cerebral binding systems have been developed with the purpose of utilizing this assay as a routine neurotoxicological test. Ligand-receptor interactions that have been characterized include those for serotonin, GABA, diazepam, glycine, muscarinic acetylcholine, dopamine, and the α and β adrenergic sites. Enkephalin receptors are also being studied. Regional distribution and specificity determinations have been made for these receptors. The developmental profile of receptors is being assayed in rats and chicks. The extent to which circadian and other environmental factors influence receptor formation and maintenance is under study.

A variety of factors which have an impact on the adaptive changes occurring in animals exposed to toxicants have been studied. Among these are: (1) effect on handling or familiarization of animals to humans upon endocrine and receptor

vectors, (2) modulation of receptor content by environmental factors relating to animal housing such as isolation or group rearing, (3) developmental neurochemical changes which may modify susceptibility to toxic agents, (4) the use of vehicles such as dimethyl sulfoxide which may in themselves be biologically active, (5) the modulation of receptor binding sites in a developing tissue after early or late deafferentation, and (6) the differential susceptibility of male and female animals to neurochemical changes induced by toxicants.

Extensive investigations on the effects of chlordecone on endocrine, neuropeptides, and neurotransmitter systems have been undertaken. Accumulation of ^{14}C-chlordecone in the CNS and pituitary precisely paralleled the distribution of ^{3}H-estradiol. Highest levels are in the pituitary, hypothalamus, and preoptic area-septum - regions important in reproductive function. Exposure of proestrus females to chlordecone (50 mg/kg in oil) leads to a rapid development of persistent vaginal estrus. Diestrus exposure produces a delayed persistent vaginal cornification occurring after the females reach the estrous stage. The delayed occurrence of persistent vaginal estrus is possibly a consequence of a block in the LH surge, but proestrus exposure does not block the immediate occurrence of the LH surge. Chlordecone mimics the effects of estrogen in increasing prolactic and decreasing luteinizing hormone in ovariectomized females, but the effect does not occur until at least 12 hours after exposure to chlordecone. Chlordecone blocks the effect of estrogen and progesterone in facilitating behavioral receptivity to a sexually active male, but estrogen's effects on vaginal cornification are not disrupted. Chlordecone not only inhibits estrogen's binding to its CNS cytosol receptor in vitro but in vivo exposure to chlordecone leads to translocation of the estrogen receptor to the nucleus.

It was reported that the pituitary [Met^{5}]-enkephalin system was regulated by estrogen. Since chlordecone exhibits estrogen-like activity, we explored the possibility that the pituitary level of [Met^{5}]-enkephalin might be altered by this neurotoxicant. A single injection of chlordecone (1 mg/pup on day 4 of age) reduced the level of [Met^{5}]-enkephalin at 70 and 120 days of age in male rats but not in females. Such treatment failed to alter the pituitary levels of β-endorphin in either sex. However, the same treatment caused a transient reduction in the hypothalamic β-endorphin level in both male and female rats without affecting the levels of other neuropeptides in the hypothalamus and other brain regions. These results suggest that hypothalamo-pituitary axis may be the primary neural target affected by chlordecone and estrogen-like activity may be related to the chlordecone-elicited decrease in pituitary [Met^{5}]-enkephalin level.

Brain levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT), and their acid metabolites such as dihydroxy phenylacetic acid (DOPAC), 3-hydroxy-4 methoxy phenyl glycol (MHPM), and 5-hydroxymelolacetic acid (5HIAA) were measured 24 hrs after a single injection of chlordecone (75 mg/kg; i.p.). Ratios of levels of metabolites over their parent amines are used as an indication of changes in turnover rates. DA system was not affected by such treatment. There was a great increase in 5-HT turnover in basal ganglia, brain stem, and hypothalamus. These results raised the possibility that the change in 5-HT system might be related to the change in body temperature or the tremor activities after chlordecone treatment. Chlordecone also caused a dramatic increase in NA turnover in hypothalamus, which was well correlated with the change in some endocrinological parameters such as growth hormone, prolactin, and leutenizing hormone. Studies are in progress in an attempt to obtain more information in correlating the changes in neurotransmitters and neuroendocrine and behavioral changes.

NEUROPHYSIOLOGY

The mission of the Neurophysiology Workgroup is to investigate the changes in neurophysiologic functioning produced by subacute or chronic exposure to low levels of various types of environmental agents. Such research may contribute significantly to environmental health sciences in at least two respects. First, it is expected that research in this area will help discover and refine methods to detect the presence of neurotoxicity following exposure to agents having direct effects on the nervous system. Second, it can aid in determining the portion(s) of the nervous system affected by neurotoxins. Thus, the neurophysiologic approach to toxicology can play an important role in guiding both neurochemists and neuropathologists with respect to the sites at which they should focus their research.

The Neurophysiology Workgroup was established this year. Initial efforts are being devoted to the effects of triethyl lead upon the functioning of certain limbic system pathways. Behavioral studies conducted in this laboratory have implicated the limbic system generally and perhaps the septum, specifically, as being the site for the neurotoxic effects of triethyl lead. Effects of triethyl lead on the threshold for chemically induced seizures, on electrically induced afterdischarges and the kindling process, and on event related electrical potentials within the limbic system are being investigated.

PERSONNEL

Additions to the Laboratory were: Staff Fellow - Dr. H. S. Swartzwelder; Staff Fellow - Dr. T. J. Walsh; Visiting Fellow - Dr. K. Yoshikawa. Individuals leaving the Laboratory of Behavioral and Neurological Toxicology were Senior Staff Fellow - Dr. P. A. Cabe; Senior Staff Fellow - Dr. R. E. Squibb; Interagency Personnel Agreement - Dr. J. A. Rosecrans.

OTHER ACTIVITIES

Dr. S. C. Bondy: Adjunct Associate Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina; Member, Editorial Board, Neurochemical Research; Member, Editorial Board, Developmental Neuroscience; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Environmental Health Sciences; Councillor, North Carolina Society for Neuroscience; Secretary-Treasurer, North Carolina Society for Neuroscience; Member, NIEHS Radiation Safety Committee; Member, Publications and Education Committee, American Society for Neurochemistry; Contributor to session on "Aluminum Neurotoxicity," Winter Conference on Brain Research, Steamboat Springs, Colorado; Contributor to session on "Effects of Glutamic Acid on the Brain, Its Relevance to the Huntingdon's Chlorea," Winter Conference on Brain Research, Steamboat Springs, Colorado; Invited seminar entitled "Neurochemistry of Lead Toxicity," British Science Association, London, England; Contributor to Roundtable on the Developmental Neurotoxicity of Lead, International Society for Developmental Neuroscience, Patras, Greece.

Dr. J. S. Hong: Adjunct Associate Professor, Department of Psychiatry, Duke University Medical School; Invited seminar entitled "Effects of Administration of Psychoactive Drugs on Brain Neuropeptide Systems," Satellite Symposium to the 8th International Congress of Pharmacology, Gifu, Japan; Invited seminar entitled "Hormonal Regulation of Pituitary Endorphin Systems," Conference on Dynamics of Neurotransmitter Function, Washington, D. C.; Invited seminar entitled "Neuropeptides

as Neurotransmitters (or Neuromodulators)," Duke University Medical School; Invited seminar entitled "Effects of Administration of Psychoactive Drugs on Brain Neuropeptide Systems," Psychiatric Research Center of Tokyo, Japan; Invited seminar entitled "Biochemical Studies of Enkephalins and Endorphins," Duke University Medical School; Invited seminar entitled "Regulation of Enkephalins in the Brain," Medical College of Wisconsin, Milwaukee, Wisconsin; Invited seminar entitled "Regulation of Brain Enkephalins by Estrogen," University of Chicago, Chicago, Illionis.

Dr. C. L. Mitchell: Adjunct Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina, lectures presented to medical, graduate and undergraduate students of the University of North Carolina; Chairman, Intramural Council, National Institute of Environmental Health Sciences; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology; Member, American Society for Pharmacology and Experimental Therapeutic's Committee on Environmental Pharmacology; Member, National Institute of Environmental Health Sciences Labor Management Committee; Member, Technical Program Committee of 1982 Annual Meeting of Bioelectromagnetics Society; Member, 9 to 11 Promotion Committee, National Institute of Environmental Health Sciences; Co-organizer, Symposium on Neurotoxicants and Adaptive Responses of the Nervous System, FASEB Annual Meeting, New Orleans, Louisiana; Co-chairman, US-USSR Workshop on Nervous System Effects of Electromagnetic Waves (0-300 GHz), Research Triangle Park, North Carolina.

Dr. H. A. Tilson: Adjunct Associate Professor, Department of Zoology, North Carolina State University; Adjunct Associate Professor, Toxicology Program, University of North Carolina; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology and Teratology; Co-organizer, Symposium on Neurotoxicants and Adaptive Responses of the Nervous System, FASEB Annual Meeting, New Orleans, Louisiana; Invited seminar entitled "Behavioral and Teratological Research in Neurotoxicology, Fall Symposium of Mid-Atlantic Chapter, Society of Toxicology, New York; Invited seminar entitled "The Neurotoxicity of Acrylamide," University of Cincinnati and Institute of Environmental Health, Cincinnati, Ohio; Invited seminar entitled "Neurotoxicity of Triethyl Lead," Satellite Symposium on Environmental Neurotoxicology - Assessment of Nervous System and Behavioral Dysfunction, Dusseldorf, Germany.

Dr. L. L. Uphouse: Member, Animal Experimental Committee, National Institute of Environmental Health Sciences; Ad Hoc Reviewer, National Science Foundation Neurobiology Program; Ad Hoc Reviewer, National Science Foundation Psychobiology Program; Ad Hoc Reviewer, National Science Foundation EPSCOR Program; Ad Hoc Reviewer, National Science Foundation Behavioral and Social Sciences; Invited seminar entitled "Chlordecone Effects on Hypothalamic-Pituitary Axis," Max-Planck, Institut fur Neurochemie, Germany.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90008-05 LBNT

PERIOD COVERED
October 1, 1981 to January 1, 1982

TITLE OF PROJECT (80 characters or less)

Acute and Chronic Neurobehavioral Toxicity of Acrylamide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS
	T. A. Burne	Psychologist	LBNT	NIEHS
	P. A. Cabe	Senior Staff Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurobehavioral Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acrylamide is an environmental toxicant capable of producing "dying back" central-peripheral axonapothies. Because acrylamide is representative of many neurotoxicants and neurological disease states, the profile of acrylamide neurotoxicity is being assessed in several behavioral procedures. Most studies are intended to provide a functional analysis of the neurotoxicity of acrylamide and to yield information concerning the functional significance of acrylamide-induced alterations in brain neurotransmitters. Other studies are desinged to investigate the toxicity of possible metabolites of acrylamide, as well as the problem of residual or masked neurotoxicity.

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

A. Previous work in this laboratory suggested that acrylamide increases affinity and/or density of dopamine receptors in the corpous striatum. The present studies investigated the functional significance of the receptor binding effects. Rats were given a dose of 100 mg/kg of acrylamide orally and challenged with 1 mg/kg apomorphine 24 hrs later. Apomorphine-induced hypermotility was significantly decreased by acrylamide pretreatment. A similar effect was observed after 10 days of dosing with 10 mg/kg/day.

B. The finding that acrylamide increases dopamine receptor binding is not easily reconcilable with the observation that acrylamide decreased responsiveness to apomorphine. One explanation of these data is that 100 mg/kg of acrylamide might have produced a peripheral effect which interferred with the expression of the apomorphine-induced hypermotility. In order to establish the specificity of the acrylamide-apomorphine interaction, psychopharmacological agents were studied. Dose response curves to the behavioral effects of apomorphine, d-amphetamine, clonidine, and chloridazepoxide were determined alone and in the presence of acrylamide using alterations in food reinforced operant behavior as the endpoint.

Rats were trained to lever touch for food on a variable interval (VI) 15 sec schedule of reinforcement. Dose response alteration of VI responding by apomorphine, d-amphetamine, clonidine, and chlordiazepoxide was studied alone and in the presence of a behaviorally ineffective dose of acrylamide. Pretreatment with 12.5 mg/kg of acrylamide 24 hrs prior to challenge with psychoactive compounds enhanced the behavioral suppressant effects of apomorphine and d-amphetamine. No significant effect of acrylamide pretreatment on the behavioral effects of clonidine and chlordiazepoxide was observed. These data suggest that acrylamide increases responsiveness to agents that act directly on dopamine (DA) receptors or indirectly by releasing DA. This change in responsiveness to apomorphine and d-amphetamine may be related to the effects of acrylamide on the affinity or density of the DA receptor. However, acrylamide did not affect the sensitivity of rats to the behavioral suppressant effects of haloperidol, a dopamine receptor antagonist, or oxotremeine, a muscarinic receptor agonist.

C. Subsequent experiments done in 1981-1982 focused on four major aspects:

1. Effects on reactivity to noxious stimuli - that acrylamide has little effect on pain transmission in humans was confirmed in our rat model. Rats receiving acrylamide daily for two weeks were found to have decreased neuromuscular grip strength, but shock titration thresholds and responses in the tail flick procedure indicated no alteration in responsivity to noxious electrical or thermal stimuli. These data support the present concept that although acrylamide affects fibers carrying sensory information, it does not affect those involved in the transmission of pain.

2. Acrylamide is extensively metabolized; less than 2% of the parent compound is found in the excreta. Studies to date have not shown convincingly that the neurotoxic effects of acrylamide are due to the parent compound or on its metabolites. Rats pretreated with ellipticine, a blocker of microsomal metabolizing enzymes, were found to be more sensitive to the neurological effects of acrylamide. Dimethyl maleate, which is a depletor of glutathione (acrylamide is metabolized extensively by glutathione S-transferase), enhanced the neurotoxicity of acrylamide. These data suggest that the metabolism of acrylamide acts to detoxify the compound.

3. One poorly understood phenomena of dying back axonapathies is the problem of residual deficits following cessation of exposure. Our data with rats suggest that rats exposed for short periods to behaviorally ineffective doses of acrylamide are more sensitive to a disruptive challenge dose.

4. Mammalian species are notoriously insensitive to the neurotoxic effects of certain organophosphate insecticides; avian species are typically very sensitive to these same compounds. Because of the demonstrated neurotoxicity of acrylamide in rats, it was of interest to determine if an avian species would also be sensitive to acrylamide. Japanese quail (Coturnix coturnix japonica) were dosed with acrylamide; at cumulative doses similar to those producing neurological signs in rats (150-200 mg/kg), acrylamide produced neurological deficits in the quail. The neurological effects were characterized as effects on righting, posture, and gait. Wing strength and tone were less affected. As in the case of mammalian species, gradual recovery of neurological function was evident following cessation of exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Agents that produce peripheral-central dying back axonapathies are encountered frequently in occupational settings. Acrylamide produces a profile of neurotoxicity in laboratory animals almost identical to that observed in humans exposed to a wide array of organic solvents. Thus, information derived from the study of acrylamide may yield clues to the mechanism(s) of a whole class of neurotoxicants (those that produce axonopathies).

PUBLICATIONS

Agrawal, A.K., Seth, P.K., Squibb, R.E., Tilson, H.A., Uphouse, L.L., and Bondy, S.C.: Neurotransmitter receptors in brain regions of acrylamide-treated rats. I. Effects of a single exposure to acrylamide. Pharmacol. Biochem. Behav. 14: 527-531, 1981.

Bondy, S.C., Tilson, H.A., and Agrawal, A.K.: Neurotransmitter receptors in brain regions of acrylamide-treated rats. II. Effects of extended exposure to acrylamide. Pharmacol. Biochem. Behav. 14: 533-537, 1981.

Cabe, P.A. and Colwell, P.B.: Toxic effects of acrylamide in Japanese quail (Coturnix coturnix japonica). J. Toxicol. Env. Hlth. 7: 935-940, 1981.

Tilson, H.A.: The neurotoxicity of acrylamide: An overview. Neurobehav. Toxicol. Teratol. 3: 445-461, 1981.

Tilson, H.A. and Squibb, R.E.: The effects of acrylamide on the behavioral suppression produced by psychoactive drugs. Neurotoxicology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90011-04 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Organometals and Related Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
Other:	T. A. Burne	Psychologist	LBNT	NIEHS
	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS
	C. F. Mactutus	Staff Fellow	LBNT	NIEHS
	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS (if any)

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	2.00	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The organometals have numerous applications in industrial and occupational settings. The neurotoxicity of these agents, particularly organoleads and tins, is well known. However, the neurobehavioral toxicity of these agents has not been studied extensively and their mechanisms are poorly understood. The purpose of these studies is to (1) characterize the neurobehavioral effects of relevant organometals, (2) assess the effects of organometals in adult, as well as developing animals, and (3) attempt to determine the site, if not the mechanism, of action of selected organometals.

PROJECT DESCRIPTION

METHODS EMPLOYED:

Functional assessment of neurotoxicity is determined using techniques developed and validated, as needed, in-house.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Triethyl tin (TET) was the first organometal chosen for study.

1. Adult Animal Model. TET bromide was found to produce dose- and time-related decreases in all neurobehavioral measures used (food and water consumption, fore- and hindlimb grip strength and startle response). Recovery of neuromuscular function was evident in animals following cessation of exposure. Animals sacrificed for neuropathological examination indicated that TET produced edema in CNS white matter. Subsequent experiments with TET in adult rats indicated that responsiveness of exposed rats to noxious stimuli remains relatively intact.

2. Developing Animals. TET bromide given in a single dose on day 4, postnatally, produced long-term alterations in neurobehavioral functioning in the absence of statistically significant decreases in body weight. These effects could be categorized as increases in motor activity and altered reactivity (impaired active avoidance, elevated latencies to emerge into novel environment). Subsequent experiments found that neonatal exposure to TET resulted in an alteration in responsiveness to the stereotypic effects of apomorphine, a dopamine receptor agonist. Postnatal exposure to TET was also found to alter the responsiveness of exposed animals in a two-choice visual discrimination task. TET-exposed rats responding on the visual discrimination task showed a greater loss of stimulus control during reversal of the contingencies than controls.

   In summary, postnatal exposure to TET produces alterations in the ontogeny of basic neurobehavioral processes, including motor activity and startle responsiveness. Alterations in the ability to adapt to novel conditions (emergence) or learning (shuttle box, discrimination reversal) situations are apparent. Changes in postsynaptic dopamine function, but not affinity or density of dopamine receptors, seems to be affected by postnatal exposure to TET.

B. Triethyl Lead

1. Adult Animal Model. The neurotoxic effects of triethyl lead chloride (TEL) were characterized in adult male Fischer-344 rats. The single dose LD50 (and 95% CI) following s.c. administration was found to be 11 mg/kg (7.6-13.3 mg/kg), while the LD50 after repeated exposure over five days

was found to be 14 mg/kg (8.3-19.0 mg/kg). TEL (1-2.5 mg/kg, s.c.) given for five days produced a phase of hyperexcitability and hyperactivity 1-2 weeks postdosing, which was followed by hypoexcitability and hypoactivity 3-4 weeks postdosing. TEL also increased hot plate and tail flick latencies during the first two weeks following TEL exposure. Subsequent experiments with an operant titration procedure indicated that TEL increased shock detection thresholds two weeks after cessation of exposure. TEL-exposed rats performed better than controls in a two-way shuttle box avoidance task three weeks after cessation of dosing. Subsequent tests indicated that flinch-jump thresholds of TEL-exposed rats were not affected. The results of these experiments indicate that TEL produces a profile of toxicity characterized by changes in reactivity or emotionality possibly similar to that of animals having lesions in limbic forebrain areas.

Subsequent experiments were designed to determine the effects of TEL on the ability of animals to withhold a response. Short-term repeated exposure to TEL impaired performance of a step-through passive avoidance task. This effect was replicated in a subsequent in which TEL was found to disinhibit punished responding in a response-contingent punishment procedure. TEL was also found to produce alterations in the ability of exposed rats to perform a complex nose poke visual discrimination task.

Further experimentation has revealed that TEL alters reactivity to noxious and non-noxious stimuli, probably without affecting detection thresholds. Neurochemical data indicate that TEL decreases concentrations of peptides in the septum and hippocampus. TEL-induced changes in reactivity to noxious stimuli are attenuated by chlordiazepoxide and higher doses of naloxone, suggesting that there may be an emotional component. Future studies will consider the following points:

(a) Increases in latencies to respond to noxious stimuli (e.g., hot plate) appear to be highly reproducible in TEL-exposed animals; pharmacological studies will be initiated to determine the possible neuropharmacological explanation for this effect.

(b) Disinhibition of responding will be characterized in other neurobehavioral tests designed to specifically detect limbic forebrain deficits, such as the radial arm maze.

(c) Direct administration of TEL into the brain and electrolytic lesion techniques will be employed in an attempt to determine more precisely the anatomical localization of TEL's site of action.

2. Developing Animals. Although alterations in development due to inorganic lead poisoning have been intensely investigated, little is known about the early toxicity of organic lead compounds. Assessment of developmental consequences due to triethyl lead (TEL) intoxication presently included (1) determination of the acute LD50 as 12.8 $\pm$ 0.9 mg/kg and (2) detailed examination of early neurobehavioral sequelae. The offspring of 12

Fischer-344 dams were administered on postpartum day 5 either a sham-injection, 15% ethanol, 3 mg/kg or 6 mg/kg TEL via s.c. injection (20 µl). Small, but significant, weight reductions for 3 (6%) and 6 (13%) mg/kg TEL-dosed pups were observed (days 14-30). Early sensory deficits of TEL pups indicated by decreased homing ability on day 7 and nipple attachment on day 9 were accompanied by the presence of fine whole body tremor (day 10). While these initial effects were transitory in nature, activity evaluations demonstrated progressive development of hypoactivity in high dose TEL males (days 15, 22, 24, 26, and 29). Passive avoidance acquisition was not affected by TEL treatment (day 18). However, 72 and 144 hr tests of passive avoidance retention (days 21 and 25) suggested alterations in low dose TEL females. A reduction in number, but not magnitude, of startle responses also occurred as a function of TEL exposure. The single bolus injection of TEL thus produced both transitory effects possibly reflecting direct pharmacological activity and potentially more permanent long-term alterations in behavioral function.

Subsequent studies of TEL in neonatally exposed animals will attempt to address the following points:

(a) Does TEL result in long-term alteration in higher order processes, such as cognition or learning?

(b) Do neurobehavioral effects correlate with blood or brain concentrations of TEL?

(c) Does TEL produce damage to limbic forebrain areas by displacing essential trace metals such as zinc, copper, or iron?

Thus far, studies have focused on triethyl tin and triethyl lead. Future studies will include the trimethyl forms of lead and tin for points of comparison.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Organometals are found with great frequency in the environment, yet there is little known about the mechanism(s) by which these agents produce their neurotoxicity. The results of our research will provide significant data on how a certain class of neurotoxicants affect neural tissue. This information will be useful in the assessment of cost-benefit questions, as well as in the development of logical strategies for the detection of toxicity and/or treatment of exposed individuals.

PUBLICATIONS

Harry, G.J. and Tilson, H.A.: The effects of postpartum exposure to triethyl tin on the neurobehavioral functioning of rats. Neurotoxicology 2: 283-296, 1981.

Harry, G.J. and Tilson, H.A.: Postpartum exposure to triethyl tin produces long-term alterations in responsiveness to apomorphine. Neurotoxicology, in press.

Squibb, R.E., Carmichael, N.G., and Tilson, H.A.: Behavioral and neuromorphological effects of triethyl tin bromide in adult rats. Toxicol. Appl. Pharmacol. 55: 188-197, 1980.

Tilson, H.A. and Burne, T.A.: Effects of triethyl tin on pain reactivity and neuromotor controls of rats. J. Toxicol. Environ. Hlth. 8: 317-324, 1981.

Tilson, H.A., Mactutus, C.F., McLamb, R.L., and Burne, T.A.: Characterization of triethyl chloride neurotoxicity in adult rats. Neurobehav. Toxicol. Teratol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90019-03 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Neurotoxicants on Neuropeptides and Neurotransmitters of Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Hong	Pharmacologist	LBNT	NIEHS
Other:	S. F. Ali	Visiting Fellow	LBNT	NIEHS
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	J. A. Rosecrans	Research Pharmacologist	LBNT	NIEHS
	H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
	L. L. Uphouse	Psychologist	LBNT	NIEHS
	W. E. Wilson	Research Chemist	LBNT	NIEHS
	K. Yoshikawa	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

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SECTION
Neurochemistry Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.30	2.20	1.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neuropeptide and neurotransmitter profiles are being examined in rats treated with various neurotoxicants either neonatally or at adult stage. Neonatal treatment with chlordecone or monosodium glutamate (MSG) produces select regional changes in [Met^5]-enkephalin and β-endorphin level in hypothalamo-pituitary axis in the adult. These results suggest that the hypothalamo-pituitary axis is very sensitive to the toxic insults. Chlordecone treatment to the adult rats also produces alterations in 5-HT and NA turnover rates on the brain which may be correlated with the changes in neuroendocrinological and behavioral changes. Lithium treatment increases substance P level in several brain regions which may be related to the change in pain threshold seen in lithium treated rats.

PROJECT DESCRIPTION

METHODS EMPLOYED:

The brain neuropeptides content is measured by radioimmunoassay methods by using specific antiserum against the neuropeptide to be measured. To enhance the specificity of radioimmunoassay, peptides are physically separated by high performance liquid chromatography before they are assayed. The levels of brain monoamines and their acid metabolites are measured by high performance liquid chromatography using electrochemical detection.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Chlordecone

1. Chlordecone (Kepone^{R}) Exposure in the Neonate Selectively Alters Brain and Pituitary Endorphin Levels in Prepuberal and Adult Rats

It was reported that the pituitary [Met^{5}]-enkephalin system was regulated by estrogen. Since chlordecone exhibits estrogen-like activity, we explored the possibility that the pituitary level of [Met^{5}]-enkephalin might be altered by this neurotoxicant. A single injection of chlordecone (1 mg/pup on day 4 of age) reduced the level of [Met^{5}]-enkephalin at 70 and 120 days of age in male rats but not in females. Such treatment failed to alter the pituitary levels of β-endorphin in either sex. However, the same treatment caused a transient reduction in the hypothalamic β-endorphin level in both male and female rats without affecting the levels of other neuropeptides in the hypothalamus and other brain regions. These results suggest that hypothalamo-pituitary axis may be the primary neural target affected by chlordecone and estrogen-like activity may be related to the chlordecone-elicited decrease in pituitary [Met^{5}]-enkephalin level.

2. Effects of Chlordecone Treatment on the Levels of Brain Monoamines and Their Acid Metabolites

Brain levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT), and their acid metabolites such as dihydroxy phenylacetic acid (DOPAC), 3-hydroxy-4 methoxy phenyl glycol (MHPM), and 5-hydroxymelolacetic acid (5HIAA) were measured 24 hrs after a single injection of chlordecone (75 mg/kg; i.p.). Ratios of levels of metabolites over their parent amines are used as an indication of changes in turnover rates. DA system was not affected by such treatment. There was a great increase in 5-HT turnover in basas ganglia, brain stem, and hypothalamus. These results raised the possibility that the change in 5-HT system might be related to the change in body temperature or the tremor activities after chlordecone treatment. Chlordecone also caused a dramatic increase in NA turnover in hypothalamus, which was well correlated with the change on some endocrinological parameters such as growth hormone, prolactin, and leutenizing hormone. Studies are in progress in an attempt to obtain more information in correlating the changes in neurotransmitters and neuroendocrine and behavioral changes.

B. Monosodium Glutamate Exposure in the Neonate Alters Hypothalamic and Pituitary Neuropeptide Levels in the Adult

Administration of monosodium glutamate (MSG) during the neonatal period in rats produced differential effects on the contents of various neuropeptides in the hypothalamus: β-endorphin (β-E) level was reduced by 70% while substance P (SP), neurotensin (NT), and Met^5-enkephalin (ME) levels were not significantly changed (ME content of male rats was slightly reduced). The contents of ME, SP, and NT in striatum and hippocampus were also unaffected by the same treatment. Male rats contain higher pituitary content of β-endorphin-like immunoreactivity (β-ELI) and abolished the sex difference in β-ELI level seen in the control rats. MSG treatment in the neonates by eliminating β-E neurons while sparing ME neurons in the brain may be a useful tool for studying the different functions of these two separate opioid peptides.

C. Effects of Lithium and Haloperidol Administration on the Brain Substance P, [Met^5]-Enkephalin Systems and on the Pain Threshold of Rats

In an attempt to obtain more biochemical information concerning the possible roles of substance P (SP) and [Met^5]-enkephalin (ME) in the etiology of mental and neurological disorders, we studied the effects of long-term administrations of lithium or haloperidol on the levels of these peptides in various rat brain regions. Daily injections of LiCl (5 meq/kg/day for 6 days) increased the SP level in regions which are enriched in dopamine innervation such as striatum, nucleus accumbens, or frontal cortex but not in the other regions such as hypothalamus, hippocampus, or brain stem. Subchronic oral administration of Li_2CO_3 through diet also caused a time-dependent increase in striatal SP level which was prevented by co-administration with haloperidol. This result suggested that dopaminergic system may mediate the change in SP level elicited by lithium. Subchronic administration of Li_2CO_3 also elevated the pain threshold which was completely blocked by haloperidol. This study illustrated the inimate relationship between lithium, dopamine, and peptide systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Evaluation of which hormones, peptides, or neurotransmitters are most easily altered in level by neurotoxicants may lead to understanding of which features of brain metabolism are especially vulnerable to chemical derangement. Such information may provide neurochemical basis for the behavioral changes after neurotoxicant treatment.

PUBLICATIONS

Hong, J.S., Lowe, C., Squibb, R.E., and Lamartiniere, C.A.: Monosodium glutamate exposure in the neonate alters hypothalamic and pituitary neuropeptide levels in the adult. Reg. Pept. 2: 347-352, 1981.

Nemeroff, C.B., Lamartiniere, C.A., Mason, G.A., Squibb, R.E., Hong, J.S., and

Hong, J.S., Majchrowicz, E., Hunt, W.A., and Gillin, J.C.: Reduction in cerebral methionine-enkephalin content during the ethanol withdrawal syndrome. Substance and Alcohol Action/Misuse 2: 233-240, 1981.

Hong, J.S., Tilson, H.A., Agrawal, A.K., Karoum, F., and Bondy, S.C.: Postsynaptic location of acrylamide-induced modulation of striatal ^{3}H-spiroperidol binding. Neurotoxicology, in press.

Hong, J.S., Yoshikawa, K., and Larmartiniere, C.A.: Hormonal regulation of pituitary endorphin systems. Raven Press, in press.

Ali, S.F., Hong, J.S., Lamb, J.C., Moore, J.A., and Bondy, S.C.: Subchronic dietary exposure of rats to chlordecone modifies levels of hypothalamic β-endorphin. Neurotoxicology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90024-03 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Chlordecone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. F. Mactutus	Staff Fellow	LBNT NIEHS
	T. A. Burne	Psychologist	LBNT NIEHS
	J. A. Rosecrans	Research Pharmacologist	LBNT NIEHS
	L. L. Uphouse	Psychologist	LBNT NIEHS
	J. S. Hong	Pharmacologist	LBNT NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chlordecone is a polycyclic chlorinated hydrocarbon insecticide known to produce hyperexcitability, tremor, and other CNS effects in exposed humans. Although chlordecone is no longer used in the United States, it is prototypic for many chlorinated hydrocarbons currently in use and/or still prevalent in the environment. The purpose of the current research project is to (1) establish suitable animal models to study the neurotoxic effects of chlordecone, (2) detect and quantify the neurotoxicity of chlordecone in adult and developing animals, and (3) attempt to determine the site, if not the mechanism(s), of action of chlordecone.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

Neurobehavioral procedures are used to assess the neurotoxicity of chlordecone. In some cases, novel instrumentation is developed to measure specific effects of chlordecone.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Adult Animals. Previous research involving repeated exposure to chlordecone via the diet indicated that the rat displayed the essential features of neurotoxicity described for humans exposed to chlordecone (hyperexcitability, short-term memory loss, tremor). Subsequent research lead to the development of a technique to quantify the tremorgenic effects of chlordecone. Tremor produced by chlordecone was found to be dose- and time-related. The onset of tremor appeared related to a threshold dose of approximately 50 mg/kg given acutely or in divided doses over 10 days. Spectral analysis of the frequency of tremor exhibited by rats exposed to chlordecone was found to be approximately 12 Hz. Chlordecone-induced tremor could be differentiated from other psychoactive agents (i.e., oxotremorine, apomorphine, harmine).

Subsequent nueropharmacological studies revealed that chlordecone-induced tremor could be blocked or attenuated by trihexyphenidyl (a muscarinic receptor blocker), mecamylamine (a nicotonic antagonist), pizotifen (a serotonergic receptor blocker), propranol (a β-adrenergic blocker), chlordiazepoxide (an anxiolytic), and muscimol (a GABA agonist). Yombine, a central α_2 adrenergic antagonist appeared to exacerbate the tremor produced by chlordecone. Although chlordecone potentiated the lethal effects of strychnine, subconvulsive doses of strychnine did not alter the dose response curve to chlordecone. Haloperidol (a dopamine antagonist), quipazine (a 5-HT agonist), and clonidine (an α-agonist) had little or no effect on chlordecone-induced tremor.

The climbing fibers of the olivocerebellar tract are necessary for the expression of harmine-induced tremor. Destruction of this pathway with 3-acetylpyridine, (3-AP) antagonized harmine-induced tremor. Tremor produced by chlordecone was accentuated, not blocked, by pretreatment with 3-AP.

These experiments suggest that chlordecone acts at spinal and supraspinal sites. The pharmacological profile exhibited by chlordecone is consistent with the involvement of a caudate-cerebellar pathway.

Chlordecone-induced hyperexcitability is also a robust neurobehavioral effect. The hyperexcitability can be quantified in terms of exaggerated startle responsiveness to air puff and acoustic stimuli. Changes in startle responsiveness appear to be associated in time with tremor. The acoustic startle appears to be mediated by a pathway having a few number of synapses consisting of auditory nerve to cochlear nucleus to the nuclei of the lateral lemniscus to the nucleus reticularis pontis caudalis. Since the startle reflex arc is well defined, the accentuated startle response produced by

chlordecone suggests that chlordecone alters the function of this multisynaptic pathway. Although chlordecone affects responsiveness to nonnoxious startle stimuli, this agent does not appear to affect responses to noxious stimuli. Alteration in shock-titration thresholds or hot plate latencies were not observed in animals showing increased startle responsiveness and tremor.

Another approach taken to determine the behavioral mechanism by which chlordecone is acting has been to study the effects of chlordecone on the hypohalamic-pituitary-adrenal axis (HPAA). Hormonal status (i.e., circulating levels of steroids) has been used as an index of arousal or response to stress, and it was postulated that chlordecone-exposed rats would have altered HPAA function. Assay of corticosertone (CS) levels following chlordecone revealed an alteration of function. Chlordecone produced an oscillating effect on CS levels over a two-week period postdosing. It is currently hypothesized that chlordecone causes an initial release of CS, which activates various feedback pathways.

Future experiments concerning the neurotoxicity of chlordecone in adult animals will continue to emphasize the possible site or mechanism of action. Several approaches, including direct application of chlordecone into stereotaxically located sites, lesioning of suspected pathways, and neurochemical confirmation will be attempted. In addition to these studies, the relationship of the chlordecone-induced hyperexcitability to tremor will be explored. Finally, the neurotoxic profiles of other chemically related agents (e.g., DDT, Mirex, Lindane) will be compared to chlordecone.

B. Developing Organism. Research in the area of developmental toxicology has concentrated primarily on the effects of chlordecone on the young animal. Initial efforts were directed at developing a set of objective and readily quantifiable tests which could be employed with the young rat. Testing was thus restricted to the latter part of the preweaning period. Moreover, the third week of life is a particularly sensitive period for evaluation of early undernutrition and stress manipulations.

1. Perinatal Exposures. Fischer-344 rats were exposed throughout gestation and the first 12 days of lactation to 1 or 6 ppm chlordecone via the maternal diet. Exposure to chlordecone did not produce maternal toxicity nor did it affect neurological functioning of the offspring at 30 or 100 days of age. Long-term alteration in dopaminergic function in males was suggested by the observation that the hypermotility produced by apomorphine, a dopamine receptor agonist, was accentuated in chlordecone-exposed animals. Subsequent experimentation with perinatally exposed animals indicated that chlordecone produces long-term alterations in the concentrations of serotonin and 5-hydroxyindoleacetic acid. Rats exposed perinatally to chlordecone displayed an apparent increase in dopamine turnover following stress. Measures of adrenal serum corticosterone (CS) indicated that chlordecone-exposed rats had CS levels that were 40-50% less than controls.

2. Neonatal Exposure. Neonatal rats were injected with 0.2-1 mg/pup on day 4 of age, as described by Gellert (Env. Res. 16: 131, 1978). Rats exposed

neonatally to chlordecone displayed a transient weight loss but no neurological deficits up to 100 days of age. Although chlordecone-exposed rats had altered rates of food-reinforced schedule-controlled responding, they did not differ in their responsiveness to d-amphetamine or apomorphine. Subsequent behavioral studies, however, indicated that chlordecone-exposed rats displayed an altered ability to reverse responding in a visually cued nose poke discrimination task.

Following the initial discovery that a single dose of chlordecone given during the neonatal period could produce long term neurobehavioral alterations, subsequent experiments were initiated. Chlordecone was administered in all experiments on day 4 postpartum at a dose of 1 mg/pup (110-120 mg/kg). The LD50 value for chlordecone via s.c. injection is 277.5 mg/kg (261.4-306.7 mg/kg). The neonatal exposure route was chosen because of the putative estrogenic effects of chlordecone and because early neonatal life is the period of neuroendocrine differentiation. The postnatal dosing regimen produces transient alterations in body weight (10-15%). Females consistently display precocial vaginal opening (as early as day 17). Delay in eye-opening, a standard morphological index of development was observed in one experiment but was not replicated in subsequent experiments.

One of the prominent effects of chlordecone following neonatal exposure appears to be some alteration in emotionality or reactivity. Chlordecone-exposed animals are hyperresponsive to a novel auditory startle stimulus; chlordecone appears not to affect the rate of habituation to repeated presentations of an auditory stimulus.

As in adults, administration of chlordecone to neonates produces a measurable tremor. One important difference between adults and neonates concerns their relative sensitivity to chlordecone. Neonates given chlordecone do not display tremors until at least 3 days postdose; adults begin to tremor as early as 1 hr postdosing. These data suggest that there is a neuronal pathway that mediates chlordecone-induced tremor and that this pathway does not appear ontogenically until at least day 7 postnatally. Subsequent experiments on the ontogeny of tremor using chlordecone and other known tremorgens are underway.

One indication of neonatal exposure to chlordecone is altered ability to perform in tasks involving an avoidance response. Alterations in the acquisition of passive avoidance, where the withholding of a response is required, were observed in chlordecone-treated pups. This impairment was most pronounced, but not restricted to, male pups. The processes of extinction and retention were altered as evidenced by behavioral tests at 72 hr and 144 hr, respectively. An increase in resistance to extinction, most pronounced in male pups, was attributed to increased responsiveness to the stressful nature of test by chlordecone-exposed pups as plasma steroids were elevated 20-40% over those of control pups. Over the 144 hr interval retention, impairment was observed in chlordecone-treated animals, again with the effect primarily noted in the male offspring.

Alterations in acquisition of active avoidance, where execution of a response is required, was also impaired in chlordecone-treated pups. This impairment was observed primarily in the female pups. After a 72 hr interval, measures of extinction, but not of retention, differentiated the treatment groups. While chlordecone treated males extinguished more rapidly than vehicle-treated controls, chlordecone treated females extinguished more slowly than vehicle-treated littermates.

To date, these studies demonstrate the subtle nature of chlordecone-induced neurobehavioral toxicity. Future studies will emphasize the conditions under which these deficits can be observed. This information will be useful in designing additional experiments to study the behavioral mechanism of chlordecone-induced neurotoxicity.

PUBLICATIONS

Squibb, R.E. and Tilson, H.A.: Neurobehavioral changes in adult Fischer-344 rats exposed to dietary levels of chlordecone (Kepone®): A 90 day of study. Neurotoxicology, in press.

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Rosecrans, J.A., Hong, J.S., and Tilson, H.A.: Approaches to studying central compensatory response to neurotoxicants through activation of the hypothalamic-pituitary-adrenal axis. Fed. Proc., in press.

Mactutus, C.F., Unger, K., and Tilson, H.A.: Neonatal chlordecone impairs early learning/retention performance in preweaning and weanling rats. Neurotoxicology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90028-03 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Assessment of the Neurotoxicity of 2,4-Dichlorophenoxyacetic Acid in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
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0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

2,4-Dichlorophenoxyacetic acid (2,4-D) is a widely used herbicide and a component of Agent Orange. The purpose of this research is to study the effects of 2,4-D exposure on the sensorimotor performance of rats, determine the time-course of neurotoxicity, and establish whether there are significant delayed neurotoxic effects after cessation of exposure.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

Male Fischer-344 adult rats are dosed orally with 2,4-D suspended in corn oil. Neuromotor performance is assessed in a battery of neurobehavioral tests.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Initial experiments were intended to provide information for subsequent dose selection. Rats dosed twice weekly with 20-80 mg/kg 2,4-D for 5 weeks showed weight loss and altered neuromuscular capacity. Fore- and hindlimb grip strength was increased following 2,4-D. Peripheral neuropathic effects, as evidenced by hindlimb splay or impaired negative geotaxis, were not observed. No changes in reactivity to non-noxious startle stimuli were observed in rats exposed to 2,4-D.

B. Because of the unusual effect of 2,4-D on grip strength, a study was designed to replicate the earlier study. Rats were dosed with 20-80 mg/kg of 2,4-D, orally, or 80 mg/kg, subcutaneously, 5 days per week for 2 weeks. 2,4-D increased grip strength for up to 6 weeks postdosing.

C. The next experiment in this series was designed to establish more precisely the time course and dose-response effects of 2,4-D on grip strength. Rats were dosed with 10-40 mg/kg of 2,4-D, orally, 5 days per week for 4 weeks. Hindlimb grip strength was increased after 1,2 and 4 weeks of dosing; forelimb grip strength was elevated after 2 and 4 weeks of dosing. The animals receiving 40 mg/kg of 2,4-D were affected more and sooner than those receiving lower doses. By six weeks postdosing, all animals receiving 2,4-D had grip strength scores similar to control rats.

These studies demonstrate that exposure to 2,4-D results in an altered neuromuscular capacity. The effect is time- and dose-related and dissipates with time following cessation of exposure. However, the nature of the 2,4-D effect (i.e., increased grip strength) is not characteristic of agents that produce peripheral neuropathies. Agents such as acrylamide and carbon disulfide produce neuromuscular weakness in screening tests; 2,4-D had the opposite effect.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

2,4-D is widely used in our environment as a potent herbicide. The effects of prolonged exposure to 2,4-D on neurobehavioral functioning and its precise effects on the nervous system needs to be systematically investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90029-02 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of Chlordecone on the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chlordecone became widely recognized as a neurotoxicant when, in 1975, several individuals received high level acute exposure in a factory in Hopewell, Virginia. We are investigating the CNS mechanisms contributing to chlordecone's production of tremors and reproductive dysfunction. The effects of chlordecone poisoning on neurotransmitter receptor binding site density, levels, and turnover; and levels of neuroactive peptides are being examined. Pituitary gonadotrophins and steroid hormone levels are being measured to assess the contribution of neuroendocrine changes to the neurotoxicity of chlordecone, and peripheral and central indices of reproductive function are being assessed. In addition, the interaction of chlordecone with the estrogen receptor is being measured and the effect of estrogen on symptoms of chlordecone neurotoxicity are being examined. Finally, the regional and subcellular distribution of chlordecone in the CNS is being studied.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

Receptor binding density is measured by binding of radioactive ligands to membranes prepared from striatal, hypothalamic, hippocampal, cortical, and cerebellar tissue. Levels of amines and turnover are measured by HPLC. Peptide content and serum levels of gonadotrophins are assessed by radioimmunoassay and levels of gonadal hormones are measured by RIA. Interaction with the estrogen receptor is evaluated by binding of radioactive estradiol or chlordecone to cystosol preparations and precipitation of the 8S receptor by protamine sulfate. Translocation by Kepone of the estrogen receptor is measured by binding of ^{3}H-estradiol to purified nuclei. Distribution of chlordecone is measured by analysis of ^{14}C-chlordecone. Sexual receptivity is evaluated by vaginal smear status and by lordosis to mount ratios.

MAJOR FINDINGS AND PROPOSED COURSE:

In the adult males, 50 mg/kg chlordecone in DMSO produces tremor indices within 5-12 hours postdosing. 100 mg/kg chlordecone produces a more rapid development of tremors which can lead to death within the next three days. With a 50 mg/kg dose, neurotransmitter receptors (e.g., serotonin, dopamine, α-noradrenergic) remain unchanged at 1, 5, and 24 hours after exposure. However, at higher doses, 5-HIAA is elevated. Prolactin levels are depressed at 1 hr after dosing but LH levels remain unaltered.

Accumulation of $^{14}_{3}$C-chlordecone in the CNS and pituitary precisely paralled the distribution of ^{3}H-estradiol. Highest levels are in the pituitary, hypothalamus, and preoptic area-septum - regions important in reproductive function. Exposure of proestrus females to chlordecone (50 mg/kg in oil) leads to a rapid development of persistent vaginal estrus. Diestrus exposure produces a delayed persistent vaginal cornification occurring after the females reach the estrous stage. The delayed occurrence of persistent vaginal estrus is possibly a consequence of a block in the LH surge, but proestrus exposure does not block the immediate occurrence of the LH surge. Chlordecone mimics the effects of estrogen in increasing prolactic and decreasing luteinizing hormone in ovariectomized females, but the effect does not occur until at least 12 hours after exposure to chlordecone.

Chlordecone blocks the effect of estrogen and progesterone in facilitating behavioral receptivity to a sexually active male, but estrogen's effects on vaginal cornification are not disrupted.

Chlordecone not only inhibits estrogen's binding to its CNS cytosol receptor *in vitro*, but *in vivo* exposure to chlordecone leads to translocation of the estrogen receptor to the nucleus.

Future work in this area will include:

A. Further evaluation of the translocation by chlordecone of the estrogen receptor.

B. Further studies of the distribution (subcellular) of chlordecone in the nervous system.

C. Interaction of chlordecone with the progesterone receptor.

D. Neurotransmitter contribution to the block of reproductive cyclicity and behavioral estrus.

E. Study of chlordecone effects on LH, LHRH, and prolactin receptors.

F. Interaction of chlordecone with adrenal steroids and their contribution to block of behavioral receptivity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE:

Because chlordecone is relatively resistant to biodegradation, its presence in the environment constitutes a continued source of exposure. In addition, chlordecone-induced tremor and hyperreactivity may be studied as a model for the neurotoxicity developed after a variety of pesticides. Furthermore, because of the probable estrogenicity of these compounds, they constitute potential risks not only for the development of tremor-like activity, but also for appropriate sexual maturation and function. Finally, study of chlordecone provides further insight into the mechanisms mediating tremor activity in general and into the consequences of interference with hypothalamic-pituitary regulation.

PUBLICATIONS

Seth, P. K., Agrawal, A. K., and Bondy, S. C.: Biochemical changes in the brain consequent to dietary exposure of developing and mature rats to chlordecone (Kepone). Toxicol. Appl. Pharmacol. 59: 262-267, 1981.

Hong, J. S. and Ali, S. F.: Chlordecone (Kepone^{R}) exposure in the neonate selectively alters brain and pituitary endorphin levels in prepuberal and adult rats. Neurotoxicology, accepted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90030-02 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Factors Influencing the Effects of Toxicants on Neurotransmitter-Related Chemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	L. L. Uphouse	Psychologist	LBNT	NIEHS
	W. E. Wilson	Research Chemist	LBNT	NIEHS

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.3	1.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several methods have been developed to allow the evaluation of neurotoxicity by testing for changes in neurotransmitter-related biochemistry in treated animals. By such approaches, the following factors have been indentified as potential contaminants or modulators of the neurochemical response: (a) Prior handling experience, (b) sex of animal; (c) the vehicle in which the toxic agent is administered, and (d) the age of the animal. Several toxic agents including manganese, acrylamide, chlordecone, and triethyl lead have been found to depress circulating testosterone suggesting the regulation of the level of the hormone is very susceptible to toxic damage. The hypothalamus seems to be more readily affected by toxic agents than other brain areas and this may account for the sensitivity of testosterone levels to change. The increased level of striatal dopamine receptors in acrylamide-treated rats has been shown to be confined to postsynaptic sites.

PROJECT DESCRIPTION

METHODS EMPLOYED:

Methods include the preparation of membrane fractions from various regions of the rat brain. These are then incubated together with a radioactive ligand specific for a given binding site and equilibration is allowed to occur. Receptor-ligand complexes are then separated from unbound labeled molecules by filtration. The extent of non-specific interactions is estimated by a parallel incubation in the presence of an excess of a non-ratioactive competing chemical.

Levels of monoamines and their catabolic products are determined by high performance liquid chromatography. Radioimmunassay of circulating hormones and cerebral peptides is routinely performed. Other laboratory methods include the capacity to assay acetylcholinesterase, DHA, RWA, lipid peroxidation, cerebral blood flow, and regional glucose consumption rates.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Development of Techniques

An HPLC system with electrochemical detector can now separate dopamine, dihydroxyphenylacetic acid, norepinephrine, serotonin, 5-hydroxyindole acetic acid, and homovanillic acid in a single run using a reversed phase column.

Radioimmunoassay techniques now allow analysis of estradiol, testosterone, prolactin, growth hormone, luteinizing hormone, and corticosterone from serum and Met^5-enkephalin, β-endorphin, substance P, and neurotensin in brain extracts.

High affinity binding methods have been developed and appropriately characterized to as to permit assay of the following receptor sites: dopamine, α-adrenergic, β-adrenergic, serotonin, glycine, γ-aminobutyric acid, muscarinic acetylcholine, benzodiazepine, opiate. It is planned to develop the assay of Na-K, ATPase sites using ^{3}H-ouabain. A paper describing a possible artifact in filtration binding studies is in press.

The potential of the retina as a target for neurotoxic studies has been evaluated. Direct intraocular injection of toxicants allows the measurement of damage to a neuronal population without hepatis metabolic modification of the toxic agent. Neurotransmitter receptor species and neuropeptides have been found not only in the neural retina but also in the pigment epithelium. The injection of acrylamide intraocularly in the chick has been found to elevate retina met-enkephalin and neurotensin levels while muscarinic receptor binding is depressed. This has been contrasted with kainic acid treatment which greatly depresses met-enkephalin and somatostatin in the retina.

B. Environmental Factors

A variety of factors have a serious impact on the adaptive changes occurring in a toxically treated animal. Manuscripts describing the following parameters are either completed or in press.

1. Effect on handling or familarization of animals to humans upon endocrine and receptor vectors.

2. Modulation of receptor content by environmental factors relating to animal housing such as isolation or group rearing.

3. Developmental neurochemical changes which may modify susceptibility to toxic agents.

4. The use of vehicles such as dimethyl sulfoxide which may in themselves be biologically active poses a problem in the interpretation of neurotoxicological related data.

5. The modulation of receptor binding sites in a developing tissue after early or late deafferentation.

6. The differential susceptibility of male and female animals to neurochemical changes induced by toxicants may be related to the endocrine system interference of some agents such as chlordecone (see also Z01 ES 90029-02 LBNT).

C. Studies on Representative Toxic Agents

1. Manganese

Six weeks of daily injection with manganese chloride (15 mg/kg body weight) caused young adult male rats to fail to gain weight normally. This treament depressed plasma testosterone and corticosterone levels but prolactin levels were unaffected. The only significant changes in the levels of a variety of neuropeptides assayed in several regions were increases in the levels of hypothalamic substance P and pituitary neurotensin. Striatal serotonin, dopamine, and their metabolites were unchanged in manganese-exposed rats relative to saline-injected controls. However, the stress of injection combined with the effect of manganese appeared to significantly increase concentrations of striatal monoamines relative to uninjected controls.

2. Acrylamide

The effect of acrylamide treatment on levels of dopamine, serotonin, and their metabolites was determined in several brain regions of the rat. Concentrations of several neuropeptides and circulating hormones were also measured. Both a single or repeated doses of acrylamide resulted in elevated levels of 5-hydroxyindolacetic acid in all regions studied (frontal cortex, striatum, hippocampus, brain stem, and hypothalamus). Changes in regional content of other monoamines were much less pronounced. Turnover studies following pargyline blockage of monoamine oxidase suggested results were due to increased rates of serotonin turnover in acrylamide-treated rats.

Changes in neuropeptide levels were only detected in the hypothalamus where a single acrylamide treatment caused elevated levels of β-endorphin

and substance P and in frontal cortex where met-enkephalin levels were higher after repeated acrylamide injection. Such repeated injection caused a major depression in plasma levels of testosterone and prolactin.

In another study, the striata of rats were unilaterally lesioned with kainic acid. After two weeks, rats were exposed to 10 doses of acrylamide (20 mg/kg body weight/dose) over two weeks. Binding of ^{3}H-spiroperidol to membranes prepared from unoperated striata was elevated in acrylamide-exposed rats relative to undosed controls. This differential was not apparent when binding was compared in membranes from kainate-treated striata of acrylamide-treated and untreated rats. A parallel acrylamide treatment of unoperated rats had no significant effect on striatal levels of dihydroxyphenylacetic acid and homovanillic acid suggesting that this neurotoxicant failed to affect the presynaptic events of the dopamine system. Thus, the alterations of the striatal dopamine receptor caused by acrylamide, that have been previously reported, appear to be confined to postsynaptic sites.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

A major feature of this work is the demonstration of the feasibility of using altered neurotransmitter-related parameters as a means of detecting neurotoxicity. We have demonstrated that relatively low doses and brief exposures to several compounds can change such characteristics. In some cases, these changes are specific for a certain transmitter species, thus offering clues as to the neuronal systems that are especially prone to toxic disruption. The emphasis on neurotransmitter features allows correlation of biochemical changes with altered behavior and comparison of effects with pharmacological agents of well-understood specificity. By this means, the sites of action of neurotoxicants can begin to be delineated. The use of several toxic agents will also help to identify those features of brain metabolism that are especially vulnerable to toxic damage. The transmitter binding screen that we are developing allows a rapid and objective evaluation to be made of the effects of a deleterious agent upon a series of neuronal pathways. The relevance of such correlations is demonstrated by the use of pharmacological challenges on exposed animals.

PUBLICATIONS

Agrawal, A.K., Squibb, R.E., and Bondy, S.C.: The effects of acrylamide treatment upon the dopamine receptor. Toxicol. Appl. Pharmacol. 58: 89-99, 1981.

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Por, S. B. and Bondy, S. C.: Regional circadian variations of acetylcholine muscarinic receptors in the rat brain. J. Neurosci. Res. 6: 315-318, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90031-01 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Assessment of Neurophysiological Effects of Organometals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
Other:	H. S. Swartzwelder	Staff Fellow	LBNT	NIEHS
	H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
	T. J. Walsh	Staff Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurophysiological Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The organometals have numerous applications in industrial and occupational settings. The neurotoxicity of these agents, particularly organoleads and tins, is well known. However, their precise sites and mechanisms of action are poorly understood. The purpose of these studies is to characterize the neurophysiological effects of relevant organometals in an attempt to determine the site of action and aid in determining the mechanism of action of selected organometals.

PROJECT DESCRIPTION

METHODS EMPLOYED:

Neurophysiological assessment is made using standard neurophysiological and electrophysiological techniques.

MAJOR FINDINGS AND PROPOSED COURSE:

Triethyl lead was the first organometal chosen for study. Behavioral studies conducted in this laboratory have implicated the limbic system as being the site for the neurotoxic effects of triethyl lead. Since damage to portions of the limbic system (e.g., hippocampus and amygdala) increases seizure susceptibility of animals, effects of triethyl lead on convulsive activity is being investigated. Preliminary investigations have shown that triethyl lead chloride (TEL) administered daily for 5 days in doses of 0.88 or 1.75 mg/kg, s.c., to adult male Fischer-344 rats increases the susceptibility to pentylenetetrazole seizures. This effect was evident for at least 4 weeks after the last dose of TEL.

Studies in progress are designed to assess the effects of TEL upon (1) hippocampal and amygdaloid afterdischarge thresholds, (2) the characteristics of threshold-level hippocampal and amygdaloid afterdischarge and their post-ictal sequelae, and (3) the rate of kindling through both hippocampal and amygdaloid electrical stimulation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Organometals are found with great frequency in the environment, yet there is little known about the mechanism(s) by which these agents produce their neurotoxicity. The results of our research will provide significant data on how a certain class of neurotoxicants affect neural tissue. This information will be useful in the assessment of cost-benefit questions, as well as in the development of logical strategies for the detection of toxicity and/or treatment of exposed individuals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90032-01 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Neurobehavioral Toxicity of Microwaves

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
Other:	H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
	D. I. McRee	Leader, Non-Ionizing Radiation Workgroup	LEB	NIEHS
	M. J. Galvin	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurobehavioral Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The problem of changes in neurobehavioral functioning following low power density microwave fields has received considerable attention, especially in Eastern European countries. These investigators consistently report animals to be less reactive to electrical shock stimuli and more prone to fatigue than control animals. Work conducted in the United States is quite controversal. Little work has been done either in the United States or in Eastern European countries on the neurobehavioral effects of perinatal exposure. The purpose of this research is to study the effects of microwave exposure perinatally on neurobehavioral parameters and work performance in rats.

PROJECT DESCRIPTION

METHODS EMPLOYED:

Pregnant female Fischer-344 rats or Japanese quail eggs are subjected to microwaves and the neurobehavioral functioning of the offspring is assessed in a battery of neurobehavioral tests developed, standardized, and validated in this laboratory.

MAJOR FINDINGS AND PROPOSED COURSE:

A preliminary study from this laboratory (Cabe and McRee, Neurobehav. Toxicol. 46: 249-256, 1980) suggested that Japanese quail exposed to 2450 MHz continuous wave microwave radiation, 5 MW/cm^2 over the first 12 days of incubation showed altered responding in a shuttle shock escape-avoidance test.

A study was recently completed in which rats were exposed to microwaves (2450 MHz, 10 mW/cm^2, 3 hrs/day) prenatally (days 5 through 20 of gestation) and perinatally (same as above plus days 2 through 20 postnatally). The animals were tested at 30 and 100 days of age in a battery of neurobehavioral tests. Assessments were body weight, motor activity, startle to acoustic and air puff stimuli, fore- and hindlimb grip strength, negative geotaxis, responsiveness to nociceptive stimuli, and swim to exhaustion. The only consistently significant effects were an increase in body weight and decreased swim time to exhaustion in the exposed animals relative to controls. The body weight increase was present at both 30 and 100 days of age; the decreased swim time was seen at 30 days of age but not 100 days of age.

Future studies will be devoted to determining the threshold for these effects and their mechanisms. Emphasis will be placed on examining neuroendocrine aspects of microwave exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Microwave exposure is pervasive in our environment. Yet little is known concerning the biological effects of exposure to low levels of microwaves, especially when this exposure occurs perinatally. Such effects need to be systematically investigated.

LABORATORY OF BIOCHEMICAL GENETICS

LABORATORY OF BIOCHEMICAL GENETICS
Summary Statement

The primary objective of the program in the Laboratory of Biochemical Genetics is focused on development of systems to monitor the human population for induction of mutations in somatic and germinal cells and to evaluate the risk of exposure of the human population to environmental pollutants. The accomplishments of the goals of the Laboratory of Biochemical Genetics are vital for the general health of the human population. In order to reach these goals it is important (1) to develop a flexible and strong inhouse research program forming teams of scientists from several different biological fields and (2) to be a training ground for established postdoctoral and predoctoral level scientists in the new techniques which are developed in the Laboratory.

INTRAMURAL RESEARCH PROGRAM

The intramural staff has been organized into two programs. The major research accomplishments in each program are as follows:

In the Mutation Monitoring Group, the mechanism for induction of mutations in mammalian cells in vivo are studied. Several different selection and detection mechanisms are used for recognizing the mutant cells. Dr. Malling has developed the technique for detection of mouse sperm which react with monospecific antibodies to rat LDH-C, indicating the presence of point mutations. This is the first time in history that anyone has detected a type of mutation in sperm which is likely to be point mutation. The frequency of this type of increase is almost proportional to the dose of procarbazine given intraperitoneally to mice. Two more compounds, ethylnitrosourea (ENU) and mitomycin-C, were tested in the LDH-C system and were both positive. Mr. Burkhart and Dr. Malling have investigated the derepression of the LDH-X gene in somatic tissue and found the frequency of mouse liver cells which expressed LDH-C increased after treatment with triethylene melamine (TEM) and ENU. Dr. Wright is in the process of producing monoclonal antibodies to LDH-C by fusion of mouse myeloma cells with lymphocytes from mice which have been immunized with LDH-C. Drs. Hessling and Malling are in the process of producing monoclonal antibodies to various alleles of the major histocompatibility locus (MHC, H-2) in mice. Using these antibodies it should be possible in lymphocytes to detect mutations in which one of the homologous genes of the MHC locus is inactivated. Measurement of the frequency of abnormal sperm is used to screen environmental pollutants. Very little is known, however, about the mechanism which results in an abnormal sperm. Mr. Burkhart and Drs. Binkert and Malling have approached this problem in two different ways: (a) by studying the inheritance of sperm abnormality and (b) by studying the induction of sperm abnormality with various mutagens. The enzyme α-glycerolphosphate dehydrogenase is bound to the mitochondrial membrane in sperm. Treatment of mice with mutagens results in high frequency of sperm without α-GPD activity. Drs. Malling and Skow and Mr. Burkhart are exploring the molecular events leading to this abnormality. The DNA content per sperm in inbred mouse strains vary very little. Dr. Malling and Mr. Burkhart are studying the variance of the DNA per sperm in progeny among mutagen-treated animals. A high frequency of individuals with increased variance of the DNA per sperm were found among such individuals. Several genetic events such as translocations were found to increase the variance of the DNA, but this could only account for a fraction of the detected individuals with higher variance.

The work of the Physiological Genetics Group is concerned with the development of methods for detecting germinal mutations and with the consequences of germinal mutations. Dr. Johnson and his colleague, Dr. Lewis, are examining progeny from mutagen-treated parents for germinally transmitted mutational events. The experimental mutagens which are used include procarbazine, methyl methane sulfonate, and ethylnitrosourea. Methods of analysis are electrophoresis, enzyme activity determinations, and other approaches. A large number of various types of mutations have been detected. These include spontaneous and induced alterations. Mutations are to be characterized as to inheritance pattern, as to their physical/chemical basis (e.g., deletions, base-pair substitutions, frameshifts), for their structural and functional effects on the gene products and according to their metabolic and physiological impact. Dr. Johnson also is studying the mutagenic effect of chemicals on morphometric characters such as bone structure. Drs. Skow and Malling have initiated a molecular analysis of the induced mutants. Drs. Skow and Johnson are developing techniques for increasing the number of markers in the biochemical specific locus system, and Dr. Skow is especially concentrating on mutations resulting in cataract mutations detected in offspring of treated mice. This last study is especially important since it directly parallels a common human detrimental genetic deficiency.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60021-10 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Investigation of Germinal Mutation Induction in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. M. Johnson	Research Geneticist	LBG	NIEHS
Other:	L. C. Skow	Senior Staff Fellow	LBG	NIEHS

COOPERATING UNITS (if any)
Research Triangle Institute, Life Sciences Group, Research Triangle Park, NC; Medical Research Council, Laboratory Animals Centre, Surrey, England

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various approaches to mutation detection are being investigated. Parental mice are exposed to mutagens such as ethylnitrosourea, and transmissible alterations in F_1 progeny are examined. Characteristics include variant enzymes detected by electrophoresis and/or change in activity, abnormalities in the skeleton, and eye defects, e.g., cataracts. Recessive effects will be examined in separate breeding studies beginning with mutagen treated parents crossing the F_1 males to untreated females (same strain as used for treatment in the first generation) and finally backcrossing the F_2 females to F_1 males.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Induced and spontaneous mutation rate data are being gathered from the mouse as alternative approaches for the detection of germinal mutation and are evaluated. Methods are based on enzyme characteristics such as activity and electrophoretic mobility. Male mice, some of which are mutagen treated, are mated with females and F_1 offspring obtained. Tissue samples are removed surgically from the parental and F_1 animals and subjected to analysis. Suspected mutants are mated to confirm the genetic basis of variants. Spontaneous and chemically induced mutants affecting phosphoglucomutases, peptidases, malic enzyme, isocitrate dehydrogenase, esterase, hemoglobin, and others have been found. Mutations that cause a reduction in activity are apparently induced to a greater frequency than those that alter electrophoretic mobility. Enzyme deficiencies in humans comprise a substantial part of the genetic disease burden. More recently the work has been expanded to include morphological abnormalities of the skeleton and defects in the eye, particularly lens cataracts. Our attempts to measure deficiencies in heterozygous F_1 offspring are probably hampered to some extent by the presence in mutant bearing F_1 of normal gene products originating from the untreated parent. Such mutants which are apparently recessive in terms of the selected observable phenotype may yet be partially dominant in their expression on other presently unrecognized characteristics and may as a result impact negatively on viability or fertility to a small but yet significant extent. A program of breeding F_1 progeny of mutagen-treated parents by a cross-backcross method has been instituted to investigate recessive effects directly and specifically.

Our general objective for the overall project is to obtain results from mice that might be helpful to understanding the impact of germ-line mutational damage to man.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The risks to human health of increasing germinal mutation rates are incompletely understood. Mutational damage is related to mutation rates and it is also determined by the specific underlying molecular mechanisms involved with exposure to particular mutagenic agents. As a variety of induced and spontaneous mutants are identified and analyzed by various criteria, more will be learned about mechanisms of actions and their biological expression in relation to health. New methods to test for mutations in the mouse hopefully will serve to predict mutational damage to humans.

PUBLICATIONS

Johnson, F. M., Roberts, G. T., Sharma, R. K., Chasalow, F., Zweidinger, R., Morgan, A., Hendren, R. W., and Lewis, S. E.: The detection of mutants in mice electrophoresis: Results of a model induction experiment with procarbazine. Genetics 97, 113-124, 1981.

Johnson, F. M., Hendren, R. W., Chasalow, F., Barnett, L. B., and Lewis, S. E.: A null mutation at the mouse phosphoglucomutase-1 locus and a new locus, Pgm-3. Biochemical Genetics 19, 599-615, 1981.

Johnson, F. M. and Lewis, S. E.: Electrophoretically detected germinal mutations induced by ethylnitrosourea in the mouse. Proc. Natl. Acad. Sci., USA 78, 3138-3141, 1981.

Johnson, F. M. and Lewis, S. E.: Mutation rate determinations based on electrophoretic analysis of laboratory mice. Mutation Res., 82, 125-135, 1981.

Johnson, F. M. and Lewis, S. E.: The human genetic risk of airborne genotoxics: An approach based on electrophoretic techniques applied to mice. In, Genotoxic Effects of Airborne Agents, ed. by R. R. Tice, D. L. Costa and K. M. Schaich, pp. 595-608, Plenum Press, NY, 1982.

Johnson, F. M. and Lewis, S. E.: Problems in genetic risk assessment: The detection of transmissible point mutations in mice by electrophoresis. In, Lectures of the Latin-American Course in Genetic Toxicology, Mexico City, August 1981, in press.

Johnson, F. M. and Lewis, S. E.: The detection of ENU-induced mutations in mice by electrophoresis and the problem of evaluating the mutation rate increase. In, Workshop on Utilization of Mammalian Specific Locus Studies in Hazard Evaluation of Genetic Risks, Research Triangle Park, NC, March 1982, in press.

Lewis, S. E. and Johnson, F. M.: Dominant and recessive effects of electrophoretically expressed specific locus mutations. In, Workshop on Utilization of Mammalian Specific Locus Studies in Hazard Evaluation of Genetic Risks, Research Triangle Park, NC, March 1982, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65007-05 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of Mutation by Using Sperm Specific Enzyme LDH-C

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. G. Burkhart	Chemist	LBG	NIEHS
Other:	M. C. Fater	Biological Aid	LBG	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.9	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was undertaken to study mutagenesis using monospecific antibodies against the sperm specific enzyme, lactate dehydrogenase-C. A monospecific antibody has been prepared from rabbit antiserum that reacts with rat sperm and not with mouse sperm. Using this antibody in a sandwich fluorescent antibody technique, point mutations in the mouse sperm were detected in mice treated with mutagenic drugs: procarbazine, mitomycin C, and ethylnitrosourea. Monospecific antibodies to mouse LDH-C which do not react with human sperm have been purified. Using these antibodies on human sperm, it may be possible to measure mutations in human sperm.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Preparation of monospecific antibody. The gamma globulin fraction from a rabbit antiserum against rat LDH-C is isolated by precipitation with 18% sodium sulfate. After dialysis against phosphate-buffered saline (PBS), the antibody is absorbed with the following materials in sequence to remove the cross-reacting antibody molecules: plain Sepharose-4B, mouse LDH-C immunoabsorbent, and mouse sperm. The absorbed preparation is checked for its monospecificity as indicated by its reaction with rat sperm and no reaction with mouse sperm.

2. Labeling technique. Fresh sperm from vasa deferens or ejaculate are washed two times with PBS and suspended in 1 ml of the same buffer. Twenty microliters of the monospecific antibody is added and the mixture incubated for three hrs at 4°C. The unreacted antibody is washed off three times with cold PBS and the sperm are again suspended in 1 ml of the buffer. Fifty microliters of a second antibody (1 mg/ml), fluorescein isothiocyanate-coupled goat anti-rabbit IgG, is then added and allowed to react for 3 hrs at 4°C. After washing and suspending, sperm concentration in the suspension is determined by the use of a hemacytometer. Fifty microliter aliquots of this suspension, containing 1-5 million sperm, are spread in the center of microscopic slides in the form of a rectangle approximately 2.5 cm x 1.3 cm in size.

3. Locating and counting the mutant sperm. The entire area of the slide containing 1-5 million sperm is scanned under a fluorescent microscope equipped with epi-illumination system. Sperm that are brightly fluorescent are counted as mutants.

MAJOR FINDINGS: The number of chemicals which are to be tested for mutagenesis in the LDH-C system have been expanded to include N,N-Bis(2-chloroethyl)-N-nitrosourea (BCNU), ethylene oxide (EO), and methyl nitrosourea (MNU). EO was administered to mice by injection and by inhalation; the other mutagens by injection. The lethality and toxicity on the male germinal tissue has been followed. The treatment with ethylnitrosourea has been repeated in order for dose relations to be established. Two different mouse strains, C57BL/6 and DBA/2J, have been injected with ENU in order to study possible differences in response to the mutagen. Antibodies to mouse LDH-C react with human sperm, and a portion of these antibodies which were monospecific for the mouse LDH-C did not react with human sperm. This may make it possible to measure mutations in humans.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies are part of the Institute's program to develop and test systems that could be used to study mutation in mammals in vivo using single cells. When fully developed, this study could be used to screen chemicals for their mutagenic/carcinogenic activity and to monitor human population for any genetic alterations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65009-04 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of DNA Variance in Sperm

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. G. Burkhart	Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. N. Benziger	Biol. Lab. Tech.	LBG	NIEHS
	F. Binkert	Visiting Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to study the induction and effect of DNA variance in male mice treated with known mutagens and in certain humans treated with therapeutic agents against leukemia and Hodgkin's disease. Some genetic events (i.e., translocations) cause abnormal sperm DNA content but there are other events in spermatogenesis such as failure of cytokinesis at first or second meiotic division or the possibility for fusion of daughter or non-daughter spermatids that may also result in sperm with abnormal amounts of DNA and an increased potential for induction of fetal chromosome aberration or spontaneous abortion. The Axiomat scanning microscope is being used to quantitate the DNA fluorescence of single sperm and spermatids. The frequency of sperm with abnormal DNA content is being measured within and between normal mice, those with known translocations, offspring of treated mice, and inbred strains with a proportion of spermatids that undergo total or partial nuclear non-disjunction during spermatogenesis. Similar techniques will be applied to normal human males and males exposed to high doses of chemotherapeutic agents.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mice have been treated with procarbazine and ethylnitrosourea in single and fractionated dosage regimens. The frequencies of sperm with abnormal DNA content is being measured at intervals after the treatment. Potentially abnormal patterns of spermatogenesis are being examined by electron microscopy of testicular section from treated mice and selected inbred strains that have high frequencies of sperm with abnormal or double DNA content.

MAJOR FINDINGS AND PROPOSED COURSE: A differentiation process resulting in binuclear sperm has been described in mice. Transmitted light and fluorescence studies of human sperm preparations suggest that a similar abnormal event may exist in humans. The frequency of diploid/binuclear sperm has been characterized in normal mice and mice exposed to procarbazine or ethylnitrosourea. There is a significant increase in mice 35 days after treatment, and at 192 days after treatment the frequency in some animals remains more than double the control level. The frequency of the sperm appearing diploid binuclear has been measured in humans and found to be higher than in mice. Sperm samples are to be obtained from males treated with chemotherapeutic drugs before or during puberty. In addition samples from human males currently undergoing treatment are to be examined. Mice exposed to ethylene oxide will also be examined and the results correlated with fertility and dominant lethal data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system may significantly reduce the time, cost, and animals needed to assess the potential of compounds to induce chromosomal damage. This work will also help in our understanding of the induction of fetal triploidy, a major cause of spontaneous abortions in humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65011-03 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Derepression of LDH-C in Mouse Hepatocytes and Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. G. Burkhart	Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. N. Benziger	Biological Lab. Tech.	LBG	NIEHS
	L. C. Skow	Senior Staff Fellow	LBG	NIEHS
	J. J. Hessling	Staff Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to develop methods to detect the rare expression of LDH-C, a sperm specific isozyme; in single hepatocytes and lymphocytes of mice. The methods are to be used to measure possible changes in the frequency of LDH-X derepression that may result from exposure to environmental agents and determine correlations between increases in somatic expression of normally repressed enzymes and mutagenic or carcinogenic events. Immunofluorescent techniques have been developed to detect LDH-C in mouse hepatocytes fixed on microscope slides. The methodology is being expanded to mouse and human lymphocytes in suspension for counting on an activated cell sorter. The frequency of hepatocytes reacting with anti LDH-C has been measured in male and female DBA/2J mice and in mice exposed to procarbazine and n-ethyl-n-nitrosourea.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary experiments indicate that the frequency of hepatocytes that react with the anti LDH-C immunofluorescent label system is 7.5-10.0 x 10^{-7} in male and female DBA/2J mice. Preliminary experiments with procarbazine and ethylnitrosourea indicate there is an increase in the frequency of hepatocytes reacting with the anti LDH-C antibody after treatment with these mutagens. An increase is still observed at 10 months after treatment. Experiments are underway to establish dose and time relationships after exposure to known mutagens. Future work will try to establish that the cells reacting with anti LDH-C are actually expressing the enzyme and the nature of the alteration causing expression. Cultures of mouse L cells have been started so that a parallel system can be developed where cells expressing LDH-C can be identified and grown in culture. These cells will be used for molecular analysis in comparison with normal pre- and post-pachytene male germ cells. Changes in the frequency of LDH-C expression that result from treatment will be correlated with data from other mammalian test systems to monitor for mutations and carcinogenesis. In addition, methods will be developed to apply automatic techniques to detect derepression events *in vitro* and *in vivo*.

METHODS EMPLOYED: Specific antibodies are raised to purified LDH-5 and LDH-C. Secondary fluorescent labels are made to the enzyme-specific antibodies. A positive response model has been developed for the presence of LDH-5 in hepatocytes (normal enzyme) that had been counted and fixed on microscope slides. The same techniques are applied with the anti LDH-C antibody; hepatocytes that express the abnormal isozyme fluoresce, others do not. The frequency of fluorescent cells is then determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a system to measure changes in the frequency of derepression of specific gene products in single cells such as hepatocytes and lymphocytes has the potential to be applied to monitoring laboratory animal and human populations for exposure to harmful environmental agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65022-02 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Types of Sperm Anomalies in Males after Treatment with a Mutagen

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Binkert	Visiting Fellow	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. G. Burkhart	Chemist	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to elucidate the connection between mutagenic treatment and type of sperm anomalies. At present different types of anomalies are tested for their usefulness in mutagenic screening. Different doses of methyl methanesulfone (MMS), ethylnitrosourea (ENU), procarbazine, ethidium bromide, and acriflavin served so far as inducer.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Basically we use sperm smears of single mice. Enzymes are detected by histochemical methods. The enzyme content will be measured by eye and by absorbance readings with a Zeiss Universal. If necessary, results will be substantiated by electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: In the study of DBA/2J mice treated with methyl methanesulfonate (MMS; 50 mg/kg, qd. x 5; 100 mg/kg, single injection and 50 mg/kg, single injection) we found that the appearance and the frequency of the different categories of sperm abnormalities were time and dose dependent. The main effect after treatment with 50 mg/kg MMS (qd. x 5) was a weakening of the connection between head and midpiece, leading to more headless sperm and single heads in the first and second week. Nearly all categories were elevated between 3-5 weeks. After treatment of 100 mg/kg MMS (single dose) we observed the following: (a) an increase in headless sperm in the second week, (b) more abnormal heads in the third week, and (c) an increase of many categories in the fourth week. Fifty mg/kg MMS (single dose) never induced any significant effect. Ethylnitrosourea (ENU), procarbazine, ethidium bromide, and acriflavin showed different patterns of time and categories of abnormal sperm. A single sublethal dose of 250 mg/kg ENU induced an overall rate of 75% abnormal sperm 28 days after treatment. Even after 192 days the overall spontaneous rate was more than doubled and many single categories more than tripled. Further studies will lead to a general evaluation schedule which is easy to handle and gives strong statements about different kinds of damage.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The tested endproduct, sperm, has the advantage to be germ-line cells, the target of most concern in the view of mutagenicity. It is also easily available in large numbers. This research project will enlarge the fundamental knowledge of the presently used sperm anomally test. Other useful sperm anomalies, besides deformed heads, e.g., mitochondrial damage, will also improve the utility and application of these easily practicable germ-line procedure.

PUBLICATIONS

Binkert, F., Burkhart, J. G., and Malling, H. V.: A new sperm abnormality test using enzymatic staining. Environmental Mutagenesis, in press, 1982.

Burkhart, J. G., Ray, C. P., and Malling, H. V.: Effect of procarbazine treatment of mice on α-glycerolphosphate dehydrogenase activity and frequency of selected abnormalities in sperm. Mutation Res., in press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65023-02 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Use of Monoclonal Antibodies to Detect Mutant Forms of Lactate Dehydrogenase-C in Sperm

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. L. Wright	Research Fellow	LBG	NIEHS
	H. V. Malling	Laboratory Chief	LBG	NIEHS
Other:	J. G. Burkhart	Chemist	LBG	NIEHS
	J. H. Swofford	Biological Laboratory Tech.	LBG	NIEHS

COOPERATING UNITS (if any)
Comparative Medicine Branch; Biometry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Our goal is to measure frequencies of point mutations in sperm from mice treated with mutagens. Our strategy for detecting mutations is based on immunologic differences in a sperm-associated isoenzyme, lactate dehydrogenase-C (LDH-C) existing as isomeric forms immunologically identifiable to each species (i.e., mouse, rat, humans). Normal antibody to rat LDH-C does not react with LDH-C associated with mouse sperm; however, low frequencies of mouse sperm contain LDH-C that reacts with antibody in rat LDH-C. Moreover, mice treated with a mutagen procarbazine generate increased frequencies of sperm that react with antibody to rat-form LDH-C. The increased frequency of mouse sperm expressing rat-form LDH-C increases linearly with increasing doses of procarbazine. Monoclonal antibodies coupled with fluorescent markers will be utilized whereby mouse sperm cells will be screened. Mutants which are identified will be sorted to confirm that variant sperm express mutant forms of LDH-C. Once the tests are validated, studies will be extended to monitor frequencies of mutant forms of LDH-C in sperm from humans with clinical histories of treatment or exposure to suspected mutagens.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenase-C (LDH-C) is an isoenzyme naturally associated with sperm of many species. Sperm from mice, rats, hamsters, and humans will be collected. From each, LDH-C will be purified and used as an immunogen and injected into mice. Our goal is to produce monoclonal antibodies to each form of LDH-C (i.e., mouse, rat, etc.). Normally monoclonal antibodies specific to rat LDH-C, for example, would not react with LDH-C associated with either mouse or human sperm. Our strategy is to utilize this antibody to detect the frequency of mutations in sperm (i.e., mouse sperm) where naturally occurring amino acids in LDH-C have been substituted by other residues to the extent that the variant form of LDH-C appears immunologically as rat-form LDH-C. Thus, the antibody to rat LDH-C binds to the variant or mutant form of LDH-C associated with mouse sperm (this variation occurs at a spontaneous frequency of 1×10^{-7}). Sperm will be collected from either untreated mice or mice treated with mutagens (i.e., procarbazine). The sperm will be combined with monoclonal antibody to rat LDH-C. Rat LDH-C antibody, bound to the variant form of LDH-C associated with mouse sperm, will be tagged with a fluorescent marker. Heterogeneous sperm populations, from either mice or humans, containing sperm either with or without LDH-C antibody fluorescein markers, will be screened for mutant sperm (containing the variant form of LDH-C). With the aid of a fluorescent activated cell sorter (FACS), variant sperm will be sorted and harvested, perhaps for breeding studies or perhaps for biochemical analysis. FACS will supply data characterizing each population of sperm. FACS will also calculate and display data relative to fluorescence polarization anisotrophy of mutant sperm populations. These data will be analyzed and plotted for recording histograms, alpha numerics or dot plots for permanent storage of information on computer discs at NIEHS and NIH. Mutations in sperm, occurring at the level where DNA translates message for LDH-C, not only will be detected by this method but a library of information will be stored concerning the frequencies of mutations at LDH-C in various species treated with mutagens.

MAJOR FINDINGS AND PROPOSED COURSE: Rat and mouse LDH-C have been purified from sperm and injected separately as immunogens into mice. Thus far mouse splenocytes have been fused with each mouse myeloma cell line SP2/O-Ag14. In addition, we have developed an enzyme immunoassay (EIA) to detect α-LDH-C activity in our hybrid cultures. The EIA detects as little as 50 picograms of LDH-C. Using this EIA, we have discovered seven hybrids which secrete antibody reactive to mouse LDH-C. Two of these hybrids have been cloned and one secretes IgG1, the other IgG2A. Both of these monoclonal antibodies to mouse LDH-C cross react with rat LDH-C. At this time experiments are in progress to produce monoclonal antibodies to rat LDH-C. Monoclonal antibodies known to be active to the isomeric form of rat LDH-C will be employed to detect frequencies of mutant forms of LDH-C (in either mice, hamsters, or humans, for example) and perhaps to biochemically analyze mutant forms of LDH-C. This will include screening sperm from mice, rats, or hamsters (either untreated animals or animals treated with mutagens) to determine the effects of mutagens on the frequencies of variation of LDH-C associated with sperm.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system is important because it will permit detection of mutational events in sperm. Moreover, this method can be expanded to screen for variation in frequencies of mutant forms of LDH-C associated with sperm from human patients with a clinical history of treatment with mutagens such as procarbazine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65024-02 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Inheritance of Different Sperm Abnormalities in the BALB/c and the PL Mouse Strain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Binkert	Visiting Fellow	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. G. Burkhart	Chemist	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to elucidate the inheritance of different sperm abnormalities found in the BALB/c and the PL mouse strain. A wide range of backcrosses were finished. The unveiling of the complex results in a blind evaluation of coded slides is currently underway.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Among the various inbred mouse strains PL and BALB/c have a high level of sperm abnormalities. The various types of sperm abnormalities are elucidated by using a stain for proteins and one for a mitochondria bound enzyme α-glycerolphosphate dehydrogenase. The mode of inheritance is studied between the mutant carrying strains on crosses and the two normal mouse strains C57BL/6 and DBA/2J and on backcrosses. Electron microscopy will be used to substantiate the findings.

MAJOR FINDINGS AND PROPOSED COURSE: The results of the earlier finished crosses were the following: The F_1 generation of crosses between BALB/c and DBA/2J showed a recessive trait with a very weak penetrance of the mutant character. The offspring of crosses between BALB/c and PL, in comparison with PL, had a reduced rate of abnormal sperm. The rate, however, was higher than among the offspring from crosses between DBA/2J and BALB/c. There was also a clear-cut maternal effect of the PL strain. A wide range of backcrosses of the F_1 generation with the parental PL and BALB/c strains are finished in the meantime as well as a blind evaluation of coded slides. An unveiling of the complex results is currently underway. Further counting or recounting will substantiate the findings.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The knowledge of the inheritance of the differentiation mechanisms in sperm is necessary for the understanding and evaluation of the different forms of sperm anomalies which occur in males after treatment with a mutagen. Afterwards a simple mutagenic screening method could be established. Sperm are easily and in great number available germ-line cells. The germ line is the target of most concern in mutagenicity testing.

The results will also lead to the explanation of certain forms of sterility in man. The knowledge of the genetic steering of the differentiation processes can later also be used for the developing of methods for birth control.

PUBLICATIONS

Burkhart, J. G. and Malling, H. V.: Sperm abnormalities in the PL/J mouse strain: A description and proposed mechanism for malformation. Gamete Res. 4: 181-183, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65026-01 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mutations at the MOD-1 Locus in Single Cells of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. G. Burkhart	Chemist	LBG	NIEHS
Other:	J. N. Benziger	Biol. Lab. Tech.	LBG	NIEHS
	L. C. Skow	Senior Staff Fellow	LBG	NIEHS
	H. V. Malling	Laboratory Chief	LBG	NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute, Research Triangle Park, NC

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to develop and validate a system for detection of induced base-pair substitutions and null mutations in single cells that has direct correlation to induction of known transmissible mutations in the biochemical specific locus test system. Specific antibodies to a somatic variant of MOD-1 induced by n-ethyl-n-nitrosourea will be produced from homozygous mice carrying the mutation. The specific marker will then be used to detect the induction of corresponding mutations in single sperm or somatic cells. Mutation frequencies will be directly compared between the transmissible germinal system and single cell system.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Electrophoretic mobility and null mutations in mice have been produced at the MOD-1 locus by mutagens and detected as transmissible mutations in the biochemical specific locus mutation system. Antibodies have been raised and fluorescence label techniques developed for MOD-1 from normal DBA/2J mice. MOD-1 will be purified from mice homozygous for a significant electrophoretic mobility alteration induced by n-ethyl-n-nitrosourea and used to raise antibodies in rabbits and mice. The antibody will then be made specific for antigenic sights of the abnormal protein MOD-1. A positive model system will be developed and tested using cells from normal mice and heterozygotes of normal/induced mutant stock. Induction of mutations in single cells that correspond to induction of the original mobility variant will be tested with known mutagens and homozygous DBA/2J mice. Induction of null mutations in single cells will be tested in heterozygous DBA/variant mice using a dual label technique with the appearance of a null defined as loss of one of the labels. Concurrently, the molecular analysis of the induced lesions can be done with tissues from existing animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Direct correlation between transmissible mutation data in whole animals and mutation data from single cell systems is complicated by the difficulty of establishing the precise genetic nature of *in vitro* single cell mutations. In addition, the types of mutations observed in a single cell system developed around natural variation may not be the same as those observed in a system based on known induced mutations. This approach will allow more direct comparison of data from single cell systems with transmissible mutation data from the biochemical specific locus approach. The approach also will provide a more knowledgeable assessment of data from single cell mutation tests in humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65027-01 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Genetics of the Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. C. Skow	Senior Staff Fellow	LBG	NIEHS
Other:	F. M. Johnson	Research Geneticist	LBG	NIEHS
	K. K. Dugger	Biological Aid	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to discover additional biochemical gene markers in the mouse that will enhance our understanding of the organization and evolution of the mammalian genome and permit further development and refinement of the biochemical specific locus test for in vivo mutagenesis studies in the mouse. A search for additional genetic polymorphisms in enzymes and other proteins is being conducted using live-trapped wild mice, interfertile subspecies of Mus musculus and a representative set of inbred strains. Protein preparations are analyzed by narrow range isoelectric focusing and other electrophoretic systems, coupled with various stains to visualize enzyme activities or protein.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mice will be received in remote isolation facility where they will be mated with C57BL/6 mice until at least 10 progeny have been weaned. Mice are then killed and various tissues removed for protein analysis. Initial efforts will concentrate on twenty enzymes which are polymorphic in humans but invariant among inbred strains of mice. As new variants are discovered, they will be placed on the C57BL/6J genetic background by repeated backcrossing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is a part of the Institute's program to further develop and refine a mammalian germinal mutation assay system, the mouse biochemical specific locus test. When fully developed, this system can be efficiently used to screen physical and chemical agents for the production of transmissible mutations. Mutants from such studies represent a unique source of material for the study of mechanisms of mutagenesis using recombinant DNA techniques.

PUBLICATIONS

Skow, L. C.: Genetic variation for prolidase (PEP-4) in the mouse maps near the gene for glucose phosphate isomerase (GPI-1) on chromosome 7. Biochemical Genetics 19: 695-700, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65028-01 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Analysis of Induced Mutations in the Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. C. Skow	Senior Staff Fellow	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. G. Burkhart	Chemist	LBG	NIEHS
	L. L. Wright	Research Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to analyze, at the gene level, a collection of germinal mutations produced by various chemicals in the course of experiments using biochemical specific locus test and other mutation assay systems. The genes to be analyzed encode LDH-C, GPI-1, MOD-1, PEP-3, and LEN-1. Multiple independently induced and/or spontaneous variants for each of these genes will be analyzed by cDNA probes produced from the normal (wild-type) mRNA for aberrant gene structure, processing, and expression.

PROJECT DESCRIPTION

MATERIALS AND METHODS: Samples of non-mutant mRNA enriched for the message of interest will be obtained by specific immunoprecipitation of polysomes and sucrose gradient purification. cDNA's will be transcribed from the enriched mRNA's and cloned via plasmids into an E. coli K-12 host. Hybrid plasmids will be screened with ^{125}I-antibody to identify those plasmids containing the cDNA of interest. The cloned cDNA's will then be used to probe the genomic DNA of mutant mice to identify the site and nature of the mutation. We also plan to use this approach to screen for hybrid plasmids containing mutated LDH-C DNA from single cell mutagenesis experiments. Mutant cDNAs will be amplified and analyzed to validate the LDH-C mutation assay system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A pressing need exists for knowledge that will permit the reasonable extrapolation of data derived from non-human mutation assay systems to the question of risk to exposed human populations. Direct extrapolation is most compelling when the mutation assay system has an identifiable, homologous counterpart in the human genome. Recent advances in our knowledge of the comparative genetics of mice and humans have identified numerous regions of the mouse and human genomes that have been highly conserved. Molecular analysis of mutations in homologous mouse genes will enable us to confidently predict the consequences of a comparable mutation in humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65029-01 LBG

PERIOD COVERED
October 1, 1981, through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Analysis of Mouse Lens Mutations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. C. Skow	Senior Staff Fellow	LBG	NIEHS
Other:	K. K. Dugger	Biological Aid	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project will provide a genetic framework for analysis of chemical or radiation-induced mutations affecting the development and function of the mouse lens. Approximately 60 mutants affecting the mouse eye are maintained in various experimental mouse colonies but few of the mutants have been characterized genetically or biochemically. A selection of mouse eye mutants has been accumulated at the NIEHS. These mutants are being analyzed by complementation analysis and linkage tests to identify loci capable of mutating to produce aberrant eye phenotypes. These stocks will then be used to determine whether induced eye mutants represent additional loci at risk or remutation at previously defined loci.

PHS-6040

PROJECT DESCRIPTION

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A knowledge of the mechanisms by which genotoxic substances interrupt normal developmental sequences is crucial to ascertain the risk associated with exposure to mutagenic and teratogenic compounds. The development and function of the vertebrate eye is exquisitely sensitive to perturbation by a variety of physical and chemical agents and the vertebrate eye, therefore, presents an excellent opportunity to investigate normal and aberrant developmental patterns. By using the numerous genetic mutants available in the mouse, we will be able to analyze developmental processes in eye formation and come to an understanding of how mutagens and teratogens disturb normal eye development.

PUBLICATIONS

Skow, L. C.: Location of a gene controlling electrophoretic variation in mouse γ-crystallins. Exp. Eye Res. 34, 1982, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65030-01 LBG

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mutation of the Murine and Human Major Histocompatibility Complex Loci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Hessling	Staff Fellow	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	L. L. Wright	Research Fellow	LBG	NIEHS
	J. G. Burkhart	Chemist	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to develop rapid and reproducible immunological and immunochemical techniques which will enable us to identify structural variants in the major histocompatibility (MHC) antigens of lymphocytes in the blood of individuals exposed to mutagenic agents. Preliminary objectives include developing a mouse animal model in which the mouse major histocompatibility antigens (H-2) may be quantitated for structural variants after exposure of lymphocytes to known or suspected mutagenic agents in vivo or in vitro and compared with the spontaneous mutation rate at these loci. Use will be made of such recent immunological advances as monoclonal antibodies against defined determinants of the MHC and the technology of rapid-flow cytometry using the fluorescence-activated cell sorter to rapidly identify and quantitate spontaneous or induced mutations at the MHC loci.

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Preparation of Monoclonal Antibody: Cell lines secreting monoclonal antibodies (hybridomas) reactive against defined determinants of murine human MHC loci are either (a) grown in tissue culture and the spent supernatant containing secreted antibody is harvested or (b) injected intraperitoneally into BALB/c mice following treatment with pristane and the ascites fluid containing secreted antibody is harvested. The gamma globulin fraction of the supernatant or ascites fluid is isolated by sodium sulfate precipitation and column chromatography. Monoclonal antibodies of certain IgG subclasses are purified by affinity chromatography with protein A-Sepharose CL4B. The antibodies may be further treated with pepsin to obtain the $F(ab')_2$ fragment in order to reduce nonspecific binding to lymphocytes. This antibody is either directly labeled with a fluorophore or used in combination with a second antibody such as fluorescein isothiocyanate-coupled goat anti-mouse IgG. 2. Fluorescent Antibody Techniques: Washed splenic lymphocytes of F_1 mice or tissue culture cell lines bearing the desired MHC determinants in haploid fashion, either of which are heterozygous at all MHC loci, are used as targets. The cells are sequentially treated in suspension at 4°C for 45-60 min with the proper dilution of two monoclonal antibodies directed against different alleles of the same MHC locus, one of which is fluorescein- and one rhodamine-labeled. The cells are then extensively washed and examined in a fluorescent microscope. 3. Quantitation of Mutation at the MHC Loci: The lymphocytes to be tested are obtained from normal, untreated mice or from mice exposed to different doses of various known or suspected mutagens in vivo. Alternatively, lymphocytes of mice or tissue culture cells are treated in vitro with these mutagens. Following the reaction with the fluorophore-labeled monoclonal antibodies, cells are quantitated for reaction with one, two, or none of the monoclonal antibodies. The very infrequent occurrence of reaction with one of the antibodies but not the other is considered to be an indication of a presumed mutation. The spontaneous or background mutation rate is compared with that in duced by mutagenic treatment. This screening procedure will, in the future, be greatly simplified by the fluorescence-activated cell sorter, which has the capacity to screen millions of cells in a very short period of time for single or dual fluorescence. Furthermore, the presumed mutant cells can be isolated and further analyzed to determine whether a heritable mutation has occurred.

MAJOR FINDINGS AND PROPOSED COURSE: Cell lines secreting monoclonal antibodies against defined determinants of the MHC loci have been obtained and antibody secreted into either the tissue culture supernatant or the mouse ascites fluid has been purified and labeled with fluorophores. Several target cell lines and the F_1 generation of several murine inbred lines, which are heterozygous at each MHC locus, have been obtained. Preliminary experiments to determine the sensitivity of the fluorescent technique will be performed; and when the detection level is such that rare mutagenic events can be quantitated, murine lymphocytes or target cell lines will be treated either in vitro or in vivo with a known mutagen, such as ethylnitrosourea (ENU) or procarbazine, and the induced mutation rate compared to the spontaneous mutation rate at the MHC locus. This course will be repeated when the fluorescence-activated cell sorter (FACS) becomes available for use. Cells detected as mutants by the FACS will be sorted out and grown in tissue culture to determine whether the presumed mutants cells are indeed structurally variant at the MHC locus due to a heritable mutation at the DNA level.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies are part of the Institute's program to develop and test systems that could be used to study mutation in mammals in vivo and in vitro using single cells and readily available somatic tissues such as blood cells. When fully developed this system could be used to screen chemicals for their mutagenic/carcinogenic activity in a mammalian system relevant to the human population, and to monitor human populations for any genetic alterations, possibly as a result of exposure to mutagenic or carcinogenic chemicals.

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

LABORATORY OF ENVIRONMENTAL BIOPHYSICS
Summary Statement

The Laboratory of Environmental Biophysics is concerned with two main areas of research: The biological effects of physical factors present in our environment (Non-ionizing Radiation, Noise, Light) and the molecular interactions that occur between environmental agents and their biological targets (Molecular Biophysics). The physical factors under current investigation include nonionizing radiation (microwaves), noise (including both auditory and non-auditory effects) and light. The Molecular Biophysics Program is mainly focused on the use of sophisticated spectroscopic techniques to monitor the interaction of environmental agents with nucleic acids, membranes, proteins and microsomal systems. The Laboratory is organized into three separate Work Groups: Nonionizing Radiation, Noise Bioeffects, and Molecular Biophysics.

NONIONIZING RADIATION

Within the nonionizing radiation program, research is being conducted to: develop microwave exposure systems for bioeffects research; develop and test techniques for measuring microwave energy absorption; determine how 2450 MHz microwave radiation interacts with biological systems at the subcellular, cellular, organ and whole animal level of complexity.

A waveguide system for exposing lobster nerves which allow for intercellular recording of action potentials during irradiation has been developed. This system has been designed so that accurate measurment of specific absorption rates (SAR's) can be made and the nerves maintained at a constant temperature during exposure. A calorimeter system for measuring whole-body SAR's has been assembled and calibrated. SAR's in rat pups ranging in weight from 5 grams to 300 grams have been measured with the system as well as SAR's in Japanese quail eggs. The circularly polarize waveguide system has been received and is in the process of being assembled.

Research into the biological effects of microwave radiation at the subcellular, cellular, and organ levels continues to be an important component of the nonionizing radiation program. Hepatic lysosomes were exposed to 2.45 GHz at SAR's of 10, 50, and 100 mW/g for 90 minutes. No effects on lysosomal fragility as determined by release of lysosomal enzymes, cathepsin D and β-glucuronidase were noted. Mitochondria were exposed to 2.45 GHz at an SAR of 100 mW/g. The data indicate that states II, III, and IV and respiratory control rates were altered. Microwave radiation at a frequency of 2.45 GHz did not affect either microtubular protein polymerization or depolymerization when measured using circular dichroism spectroscopy during exposure to SAR's up to 100 mW/g. The binding of calcium to erythocyte ghost was not changed by 2.45 GHz microwaves at SAR's up to 200 mW/g as measured by spectrophotofluorimetry techniques during exposure. Isolated frog sciatic nerves were exposed to 2.45 GHz microwave radiation, sine-wave modulated at 8, 16 and 32 Hz. The nerves were exposed to SAR's of 10 and 50 mW/g. A loss in vitality occurred at 50 mW/g but not at 10 mW/g for all modulation frequencies. These results suggest that the loss in nerve vitality is nonlinear with respect to microwave intensity since CW and pulse microwaves produced the effect at an SAR of 10 mW/g. This type of nonlinear behavior would be expected if the neural membrane is acting as a diode-like detector of the microwave field. Mature spermatocytes from turkey semen were exposed for 30 minutes to 2.45 GHz microwaves at SAR's of

10 and 50 mW/g. Microwaves did not alter membrane permeability to a vital stain or the intracellular enzymes LDH and GOT. The in vivo performances of the sperm following in vitro irradiation was also examined. The fertility of virgin turkey hens given a single insemination of microwave exposed sperm was similar to the nonexposed sperm. Hatchability and embryonic mortality were not different among the treatment groups. Hematological changes in the turkey poults from eggs fertilized by exposed sperm were observed. In the group exposed to 50 mW/g a significant decreases in hematocrit and lower numbers of RBC's were measured at 2 and 4 weeks of age. At 5 and 22 weeks of age the differences had vanished indicating a compensatory characteristic with increased age.

A significant effort has been made to determine the teratogenic and developmental effects of 2.45 GHz microwave radiation. Studies examining the effect on brain development have been completed. Pregnant rats were exposed to 10 mW/cm^2 (SAR = 2 mW/g) for 3 hours per day from day 4 of pregnancy and exposure of the offspring to 10 mW/cm^2 was continued through 40 days post partum. No significant effect on brain development was observed from histological examination. However, when some of the rat pups were tested in a swim test, the exposed rats has a significantly shorter swim time to exhaustion than the control rats. Brain development in Japanese quail embryos exposed to 5 mW/cm^2 (SAR = 4.03 mW/g) 24 hours per day from day 1 to day 12 of development was also studied. A slight but statistically significant retardation was found in the development of the cerebellar cortices at days, 12, 13 and 14 of development. Some quail were allowed to hatch and were examined at 8 weeks of age. No significant differences were noted between irradiated and control cerebella at this time. Other Japanese quail eggs were subjected to 2.45 GHz CW microwave radiation at 5 mW/cm^2 (SAR = 4.03 mW/g) during the first 12 days of embryogeny. Following hatching the exposed embryos, as well as nonexposed controls, were reared to 22 weeks of age. Humoral immune potential, as indicated by comparable anti-CRBC antibody, IgM and IgG, levels at 0, 4 and 7 days post-immunization in both exposed and control quail, was not affected significantly. However, cell mediated immune potential measured by the reaction to introdermal injection of phytohemagglutinin-P in the wing web, was reduced in the exposed females, but not in the exposed males. Additionally, total leucocyte numbers and absolute circulating numbers of lymphocytes, monocytes and heterophils were increased significantly only in the exposed females. These data show that exposure of Japanese quail during embryogenesis reduced cell mediated immune potential and induced a general leucocytosis in females.

NOISE BIOEFFECTS

The Noise Effects Workgroup conducts research to: identify the physiological, biochemical and structural mechanisms that lead to cellular and neural damage associated with permanent hearing loss when activated by exposure to excessive noise or ototoxic agents; identify those environmental agents, drugs, etc. that potentiate hearing loss from noise exposure and to characterize the degree and extent to which the additional hearing loss occurs; study those specific non-auditory systems (endocrine, immunologic, physiologic, pharmacologic, teratogenic, cardiovascular) that may be affected by chronic noise exposure; identify, by appropriate epidemiological methods, those factors that are related to hearing loss and the non-auditory effects of noise.

Areas under current investigation include: investigation of the correlation between the cochlear potentials and ion movements within the cochlea and their alteration by exposure to noise; examination of the quantitative differences between damage caused by impact and steady state noise when equated for total

energy content; studies on the physiological mechanisms underlying the cochlear damage caused by cis-dichlorodiammine platinum (II); identification of the physiological mechanisms corresponding to complex signal analysis (speech) breakdown after slight noise trauma not predictable from simple signal pure tone tests; verification of technical factors affecting the precision of current speech discrimination tests and the development of electronic hardware to correct some of the sources affecting the precision; and noise effects upon the embryo and fetus.

The studies on the role of ionic permeability of the endolymph-perilymph barrier in normal and noise exposed guinea pigs have continued. Exposure to noise caused a decrease of K^+ permeability in the cochlear partition which was clearly correlated with the suppression of hair cell activity. Exposure to noise was found to alter the K^+ permeability of the organ of Corti selectively. These results were further supported by our findings that intracochlear application of tetraethylammonium suppressed hair cell function. Using intracellular recording of hair cells of the organ of Corti, it was found that maintenance of the hair cell function was dependent on the K^+ conductance of the apical membranes of hair cells. The endolymph-perilymph barrier was found to be less permeable to Na^+ than to K^+. Exposure to noise did not result in substantial changes in Na^+ permeability of the endolymph-perilymph barrier. The movement of Cl^- in the cochlear fluids was coupled with entry of K^+ into the endolymph so as to maintain electroneutrality of the endolymph. The rate constant for water of the endolymph-perilymph barrier was also examined in normal guinea pigs in conjunction with measurements of osmolarity of the cochlear fluids. The preliminary results showed that the rate constant for water was substantially greater than that for K^+ or Cl^-.

The effects of continuous and impact noise on cochlear potentials were compared in guinea pigs. Our results indicated that the degree of cochlear response suppression produced by impact and continuous noise of equal energy was not equivalent under short-term exposure conditions. The degree of cochlear response suppression produced by long-term exposure will be studied in guinea pigs with chronically implanted round-window electrodes.

The ototoxicity of cis-dichlorodiammine platinum (II) was studied in guinea pigs. Multiple administration of cis-DDP resulted in a marked suppression of the cochlear microphonics but little change in the endocochlear potential was observed. The suppression of hair cell activity was more pronounced in the lower turns of the cochlea. There was a close correleation between loss of hair cells and suppression of cochlear microphonics. No marked changes in electrolyte concentrations in the cochlear fluids were observed in cis-DDP treated guinea pigs.

An electronic auditory nerve simulator has been constructed for use in shakedown and testing of various strategies for computerized for generating trinary pseudo random noise was developed, since the binary noise conventionally used to test auditory system transfer function was recently shown to generate 2nd order distortion of the same type as the auditory system. A general purpose (computer based) transfer function analysis program accepting analog inputs while outputting transfer function magnitude and phase, cocherence, input and output power spectra, auto and cross correlation and impulse or step response has been developed. Extension of the transfer function program to accept nerve fiber histogram outputs is being pursued.

Experiments to determine the effect of *in utero* noise exposure on the conceptus and fetus have continued. The exposure of pregnant CF-1 mice to either semi-continuous high level noise (126 dBA jet engine noise) or unanticipated high

intensity startling sounds, resulted in embryo-lethality and decreased pregnancy maintenance. This effect did not appear to be related to elevation of plasma coritcosterone levels. No teratogenic effects were noted in CF-1 mice in response to noise exposure. Exposure of the CF-1 mouse to noises, whose spectra were coincident with the most acute frequency band of the mouse (18-20 KHz), resulted in significant late stage fetolethality, an effect previously shown by others to be correlated with exogenous catecholamine application. Replication of the experiment in CD-1 mice with concomitant measurement of catecholamine levels in plasma and uteri failed to generate late stage fetolethality. Teratogenic effects, lowered pregnancy rate, excess early stage resorption, and lowered maternal and fetal weight were noted. Plasma norepinepherine/epinepherine ratio was reversed by noise exposure in late stage pregnancy and uterine norepinepherine levels were significantly elevated during late stage pregnancy in noise exposed CD-1 mice. This experiment also revealed a previously unreported universe relationship between uterine NE content and plasma corticosterone and progesterone concentration during the postimplantation period. In another experiment exposure of pregnant guinea pigs to textile mill noise (elevated to 115 dB SPL) caused a significant deterioration ring of the offspring as measured by the brainstem evoked response techniques.

In conjunction with the Laboratory of Behavioral and Neurological Toxicity, the effects of maternal noise exposure on neurobehavioral parameters during adult life were assessed. Maternal exposure resulted in neuromotor (grip strength, swim endurance) differences in both male and female offspring as adults. An effort to identify the mechanisms involved in this result is underway.

A version of a fiber optic lever optimized for impact measurments of mechanical surfaces has been developed. This version, which should be very useful in diagnosing noise emission from complex machinery, is being used by others as the vibration sensing component of an acoustic intensity meter. Fiber optic motion sensor technology intended for use in investigating basilar membrane signal conversion characteristics using a twist-etch bottle coupler terminated in a single 50 μ fiber is being pursued. Techniques for fiber etching, sputtering gold film on the 45° fiber tip, and acquiring sufficient bandwidth from a one nanowatt optical return signal have been developed.

As part of an investigation into factors affecting precision of current speech-perception-in-noise tests, an instrument was developed which measures all relevant speech electro-acoustic amplitude parameters. Since provision for generating and modulating white, pink, and speech spectrum noise was also included, the instrument can be utilized to investigate interaction of the masking noise spectra characteristics and intelligibility; including the potential for confounding test scores. Since the major technical capabilities have been assembled in a single instrument for the first time, this development is expected to stimulate further investigation, perhaps leading to progress in assessing the social and economic cost of hearing loss. Progress in quantifying hearing loss relative to the difficulties experienced in everyday noisy environments is presently impeded by the imprecision of conventional methodology and technology. The instrument has been used in this laboratory in assessing intelligibility of speech passed through hearing aids and novel speech transduction devices having less distortion. In these tests, scores obtained with speech mechanically coupled onto the ossicular chain and electronically recovered from the cochlea by differential electrode techniques significantly exceeded those obtained with similar speech passed through hearing aids. Additional tests showed that, where distortion of the peak clipping type was involved, the conventional speech peak equalization methodology

acted to confound test scores while use of the long term RMS amplitude measure did not.

The technology and experience gained in these tests are being further exploited through recording of test tapes designed to quantify the potential for noise spectra and intermodulation distortion confounding. Data acquisition from normal and hearing damaged groups is to be obtained by others colaborating with this laboratory.

LIGHT

This program is concerned both with the biological effects of artificial lighting and with the interactions that occur between light and chemical agents in the skin (photosensitization). The beneficial effect of sunlight in the photoactivation of vitamin D precursors in the skin is well known. Cyclical changes in lighting also affect the maturation of gonads in both mammals and man. In addition to these effects sunlight elicits a number of undesirable side effects ranging from erythema ("sunburn") to skin cancer. More recently it has been suggested that artifical light sources, particularly those which have energy spectra that are markedly different from sunlight, may have undesirable side effects. For this reason, we have carried out a series of studies to determine whether fluorescent lights and high pressure sodium vapor (HPSV) lamps have any hitherto unknown biological effects. Sprague-Dawley rats were born and reared under either daylight-simulating fluorescent lights or HPSV lamps. Animals housed under the HPSV lamps had heavier adrenals, smaller gonads (males only), larger kidneys (females only) and elevated red and white cell counts. No differences were observed between the two groups in the swim endurance, tail flick and hotplate tests.

Another adverse effect of light results from its interaction with chemical agents in the skin. The chemical agent may be endogenous (e.g., protoporphyrin), a drug (e.g., sulfonamides, declomycin), topical agent (e.g., p-aminobenzoic acid in sunscreens) or an environmental agent (e.g., polycyclic aromatic hydrocarbons). The combined effect of light and these agents causes skin photosensitization which may take the form of either phototoxicity or photoallergy. While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanism is unknown. Irradiation of several sulfonamide drugs in aqueous and organic solution has been shown to produce a variety of carbon, sulfur and nitrogen-centered free radicals. Data suggest that the sulfur trioxide anion radical ($SO_3^{\cdot -}$) may be involved in the photosensitivity induced by these compounds. The phototoxicity of the anti-inflammatory drug benoxaprofen appears to result from the generation of oxygen-centered reactive species including superoxide, singlet oxygen and peroxy radicals. Irradiation of methanolic solutions of musk ambrette (2,6-dinitro-3-methoxy-4-t-butylbenzene) generated two nitro anion radicals that further decompose to other photoproducts.

The photodegradation of polycyclic aromatic hydrocarbons absorbed onto solid phases is the subject of a grant. In this work, the photochemistry of particulate adsorbed polycyclic aromatic hydrocarbons is being examined in a fluidized bed reactor irradiated by a xenon arc lamp. In this system the half life of pyrene adsorbed to glass particles is about 160 minutes. Six different pyrene photoproducts have been isolated using high pressure liquid chromatography.

MOLECULAR BIOPHYSICS

The Molecular Biophysics Program is conducting research to understand at the molecular level the interaction of environmental agents with target biological systems including nucleic acids, proteins membranes and microsomal systems. For these studies a number of highly sophisticated spectroscopic techniques, e.g., electron spin resonance and nuclear magnetic spectroscopy, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry, are being employed.

Nucleic Acids:

It is now widely recognized that many mutagenic agents exert their biological effects by modifying DNA. The covalent binding of chemically reduced adriamycin and daunorubicin to DNA has been examined. Results show that, under identical conditions, one adriamycin molecule is bound per 15 nucleotides whereas only one daunorubicin is bound per 140 nucleotides. These findings may explain why adriamycin induces more DNA damage than daunorubicin as evidenced by an increase in sister chromatid exchange. Enzymatically activated drugs also bind covalently to DNA with identical binding ratios. Results with synthetic polynucleotides show that the binding takes place predominately at guanine bases of DNA. In vivo binding studies show that adriamycin binds to DNA, RNA and proteins, however, binding decreases rapidly with time suggesting an enzymatic repair process is operative. The active alkylating agents derived from these drugs are not known at this time. Other investigations have also shown that several antitumor drugs induce membrane protein conformational changes in erythrocyte ghosts and mastocytoma cells. This finding suggests that the cytotoxic and mutagenic properties of these agents may involve membrane effects as well as interaction with nucleic acids. Recently, Moore has proposed a mechanism for the bioactivation of adriamycin to a covalent binding species. In this scheme, a quinone methide with a carbocation character at C_7 is presumed to be alkylating species. We had earlier suggested that, in addition to the quinone methide, a C_7 free radical intermediate may also act as an alkylating agent. We have recently examined the formation and binding of these species from adriamycin and daunorubicin to DNA. Our studies indicate that the microsomal -NADPH activation produces both one electron (C_7-free radical) and two electron reduction (C_7-quinone methide) products from adriamycin and daunorubicin. Furthermore, 105,000 g supernatant produces only the two electron reduction product. We have further shown that both one electron reduction product and two electron reduction product alkylate DNA.

Superoxide ($O_2^{\cdot-}$) or its toxic metabolites formed from the microsomal-NADPH incubations of anthracyclines have been recently proposed to act as primary toxic agents. Furthermore, these species have also been implicated in the cardiotoxicity of anthracyclines. We have examined the ability of six anthracyclines and DHAQ, (a related anthraquinone compound with demonstrated antitumor activity and cardiotoxicity) to stimulate $O_2^{\cdot-}$ production in rat hepatic microsomes and beef heart submitochondrial particles. Our studies indicate that antitumor activity of anthracyclines is not related to $O_2^{\cdot-}$ production. In addition, intercalated drugs are not effective in $O_2^{\cdot-}$ production and hence cannot as carriers for site specific production of $O_2^{\cdot-}$. Our studies also show that these agents induce peroxidation of rat hepatic microsomal lipid and that highly cardiotoxic agent induce significantly more lipid peroxidation than those agents that are less cardiotoxic. Thus it appears that lipid peroxidation plays an important role in the pathogenesis of

cardiotoxicity. Further, α-tocopherol, a known free radical scavanger, offered only a marginal protection against lipid peroxidation induced by these agents. Reduced glutathione, on the other hand afforded a complete protection against lipid peroxidation.

Membranes:

A large number of chemical agents are known to cause mast cell degranulation with the concomitant release of histamine, serotonin and other pharmacologically active compounds. While the initial event must involve the binding of these agents to the mast cell membrane, little is known about the molecular events that ultimately lead to degranulation. The interaction of compound 48/80 (a classical mast cell degranulating agent) with a number of membrane systems (erythrocyte ghosts, mastocytoma cells, purified mast cells) has been studied in an attempt to elucidate the mechanism of histamine release. The fluidity of the mastocytoma cell membranes was found to be significantly greater than that of normal mast cells. Compound 48/80 did not affect the fluidity of the membrane but did increase the number of binding sites available for spin-labeled stearic acids. While other experiments suggested that 48/80 induced a conformational change in the membrane proteins of erythrocyte ghosts, the significance of this finding and its relevance to the effects of this agent on mast cells is unclear. With the aid of fluorescence microscopy it has been possible to show that, at low concentrations, 48/80 binds to sites in the cell membrane of most cells and mastocytoma, while at high concentrations binding also occurred to intracellular anionic sites (DNA in the mastocytoma cells and granules in mast cells).

The effect of 2450 MHz microwave radiation at 8.2 mW/cm^2 and 41 mW/cm^2 SAR (specific absorption rate) on mast cells has been studied. Histamine secretion was stimulated via three different biochemical pathways using compound 48/80, concanavalin A and ionophore A23187. The results showed that microwave radiation did not affect mast cells or histamine secretion induced by any of these stimulators. It has been suggested that microwave may induce local temperature gradients across cell membranes. By measuring heat-induced inhibition of histamine secretion, we have shown that, under our exposure conditions, the cells were heated between 0.4-0.9°C above ambient temperature when exposed for 10 min at 41 mW SAR.

Mast cells have been used as a tool to study protoporphrin-induced phototoxicity in a eukaryotic cell. It has been found that 48/80 induced histamine secretion is inhibited in the presence of protoporphrin and low intensity light. The inhibition may be due to crosslinking of membrane proteins caused by photoxidation of susceptible amino acid residues. This inhibition is irreversible and, once initiated, continues in the dark although it does not develop into lysis. In contrast, protoporphrin can induce lysis in mast cells under the influence of high intensity light. The molecular basis for this effect and presumably the phototoxic inhibition of secretion may be similar to that described for erythrocytes under the influence of erythropoietic protoporphoria.

Circular dichroism studies of erthrocyte ghost membrane proteins have shown a significant decrease in the optical rotation at 224 nm after protoporphrin and high intensity light exposure. This indicates that protoporphrin phototoxicity causes a decrease in the α-helical structure of the membrane proteins. This technique has made it possible to study more precisely the development of protoorphrin phototoxicity in erythrocyte membranes and the molecular pathways involved.

Anthracene phototoxicity is also being investigated. Erythrocytes exposed to anthracene show up to 78% hemolysis after 90 min illumination. The effect is concentration and time dependent and does not occur in the dark. Recent experiments, using anthracene-derivatized fatty acids, have shown that anthracene is much more toxic to erythrocytes when covalently bound close to the polar end of the fatty acid molecule (2-anthroyl-palmitic acid). Anthracene attached to the 16th carbon of palmitic acid was not effective as a photosensitizer. These data indicate that the site of photosensitization by anthracene is near the interface region of the erythrocyte membrane. Future plans include circular dichroism studies of anthracene and anthracene-derivatized fatty acids and the effect of D_2O. The phototoxic properties of anthracene and anthracene-derivatized fatty acids will be investigated using the rat peritoneal mast cell system.

The effect of 2450 MHz microwave radiation on the membrane proteins of human erythrocyte ghosts has been investigated using ultraviolet circular dichroism spectroscopy. A specially constructed waveguide inserted into a spectropolarimeter allowed continuous recording of the optical activity due to secondary structure in membrane proteins. Microwave-induced conformational changes in protein α-helical structure have been compared with the effects of conventional heating.

The ultraviolet circular dichroism data indicate that high levels of 2450 MHz microwave radiation (600 mW/g, specific absorption rate) induce decreases in α-helical conformation that may be due both to thermal vibrations and to increased strain on the intramolecular hydrogen bonds that maintain secondary structure. The latter effect may result from differential intramolecular interactions with the oscillating electric field. Spectrin (bands 1 and 2) isolated from the ghosts was more sensitive to microwave irradiation than intact ghosts, and spectrin-depleted vesicles were the least sensitive. The data, therefore, indicate that the α-helical conformation of spectrin is altered by high levels of microwave radiation.

The effect of 2450 MHz microwave radiation on erythrocyte membrane protein conformation and calcium binding was studied with the fluorescent probe, 1-anilino-8-naphthalenesulfonate (ANS). Using fiber optic cables, excitation light was delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a fluid-filled, temperature-controlled waveguide. Fluorescence was collected using an identical cable and transferred through appropriate filters to standard detecting, amplification and recording devices.

Microwave radiation at specific sbsorption rates of 10 and 200 mW/g had no effect on the binding of the fluorescent probe, ANS, to the erythrocyte membranes. Concentration response curves of increased fluorescent intensity versus calcium concentration also showed no microwave influence on calcium binding between 2.0 - 10.0 x 10^{-4}M. In addition, experiments studying fluorescence energy transfer between intrinsic tryptophan residues and membrane bound ANS showed that intermolecular distances between donor and acceptor were also unaffected by microwave radiation.

Proteins:

Binding of metal ions Cu^{2+} and Ni^{2+} to albumins from different animal sources were studied using several physical techniques. The results from chloride ion NMR probe experiments are in good qualitative agreement with our previous studies using a cupric ion selective electrode. The chloride ion NMR studies seem to suggest a strong similarity between the Cu^{2+} and Ni^{2+} binding to different

albumins and the relatively high motional freedom at the N-terminal copper site. The circular dichroic spectra observed below 600 nm for Cu^{2+} binding to human serum albumin (HSA) were absent in the case of Cu^{2+} binding to bovine serum albumin. The binding site in HSA composed is of 4 nitrogen ligand in the plane of the Cu^{2+} complex.

More than 12 histidine C-2 and C-4 protons were observed in the 400 MHz proton magnetic resonance spectrum of HSA. Attempts will be made to make individual assignments to the specific His residues. It has been possible to label HSA with 2,6-dinitro-4-trifluoromethyl benzene sulfonyl group and observe the ^{19}F NMR spectrum of the bound label. Further studies are being made with the labeled protein. The label is presumed to be at the acetylation site of HSA by aspirin. Fluorescence spectroscopic studies have been made with modified HSA. The results obtained so far show that the fluorescent label at the lone sulfhydryl group, the bilrubin binding site and the lone tryptophan are so oriented that from the energy transfer it is possible to make some critical distance measurements. Circular dichroism studies showed induced dichroism for the bound label indicating a chiral environment. A tetrapeptide with Asp-ALa-His-Lys-OMe has been synthesized. Efforts are being made to characterize and use it as a model for N-terminal Cu^{2+} site of albumin.

Microsomal Systems:

It is now generally accepted that the toxicity of many xenobiotics results from the generation of highly reactive chemical species including free radicals. The aims of this project are to: discover new free radical pathways of metabolism; investigate the subsequent reactions of these free radicals with macromolecules and oxygen in order to understand their biochemical fate; develop approaches that permit the clear delineation of the role of free radicals in xenobiotic toxicity. Three new classes of free radical metabolites have been discovered: semiquinone-imine radicals (actinomycin D, dichloroindophenol and serotonin), triphenylmethyl carbon-centered radicals (gentian violet) and sulfur dioxide-derived radicals ($SO_2^{-\cdot}$ and $SO_3^{-\cdot}$). In addition, a number of free radical reactions with direct biochemical consequences have been examined. These include: the oxygen inhibition of nitro and azo reductases and the associated superoxide formation; the oxidation of NADH to a free radical by the serotonin semiquinone-imine; the formation of a polymeric material from anthracycline free radicals which has an electron spin resonance spectrum like melanin; the oxidation of hydralazine by metal ions or red cells to nitrogen-centered hydralazyl radicals. Finally, a new type of metabolite, identified as an arachidonic acid adduct of nitroso compounds, has been discovered during an investigation of the mechanism of prostaglandin synthetase. Prostaglandin synthetase has a peroxidase activity which appears to catalyze free reactions identical to those catalyzed by horseradish peroxidase. The free radical intermediates of benzidines, hydrazines, aminopyrine, acridine and SO_2 have been studied in these systems.

Personnel

The laboratory is organized into three separate Work Groups: Non-ionizing Radiation (Dr. Donald I. McRee, Head), Noise Bioeffects (Dr. Colin F. Chignell, Acting Head) and Molecular Biophysics (Dr. Colin F. Chignell, Head). The Molecular Biophysics Workgroup had several new additions: Dr. Krzystof Reszka (Visiting Fellow) in February, 1982, Dr. Ann Motten (Staff Fellow) in June, 1982, Dr. Roberto DoCampo (Visiting Associate), in July, 1982, Dr. Sylvia Moreno (Visiting Fellow)

in July, 1982, Dr. Volker Fischer (Visiting Fellow) in July 1982 and Dr. Robert Hall (Staff Fellow) in September, 1982. The following Guest Workers also spent time in the Laboratory, Dr. Paul West (University of Victoria, B.C.), Dr. Thomas Ade (University of Tübingen, FRG) and Dr. J. Hwang (University of Petroleum and Minerals, Saudi Arabia). Dr. Hirohiko Mori (Visiting Associate) left the Noise Bioeffects Workgroup in June, 1982 to return to Japan.

Dr. Colin F. Chignell: Adjunct Professor of Pharmacology, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, NIEHS representative, US-USSR Cooperative Program on Photobiology; member of American National Standards Institute Z-311 Committee on the Biological Effects of Nonionizing Radiation. Managing Editor, Journal of Biochemical and Biophysical Methods; Editorial Board member: Molecular Pharmacology, Proceedings of the Society for Experimental Biology and Medicine, Environmental Health Perspectives, Chemico-Biological Interactions. Invited speaker at the International Symposium on Spin Trapping and Nitroxyl Radical Chemistry, Guelph, Ontario, Canada, July 12-18, 1981.

Dr. Michael J. Galvin: Adjunct assistant professor, Department of Poultry Science, NCSU, Adjunct Assistant Professor, Department of Physiology, Duke Medical School. Participant, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation. Technical program committee, Bioelectromagnetics Society, and membership committee, Shock Society.

Dr. Teruzo Konishi: Invited speaker to the Association for Research in Otolaryngology Workshop on "Unresolved Research Problems in Menière's Disease" (January 1982).

Dr. Ronald P. Mason: Adjunct Associate Professor of Toxicology, School of Medicine, University of North Carolina at Chapel Hill. Invited speaker American Association for the Advancement of Science, Toronto (1981); Biophysics Department Roswell Park Memorial Institute; Radiation Laboratory, University of Notre Dame; National Biomedical ESR Center, the Medical College of Wisconsin; Laboratory of Toxicology, Harvard School of Public Health; Imperial Chemical Limited, Manchester, England. Invited speaker at meeting on "Free Radicals, Lipid Peroxidation and Cancer", London, England, (July, 1981); Magnetic Resonance in Biology and Medicine Gordon Conference, Tilton (August, 1982); CIIT symposium on "Toxicity of Nitroaromatic compounds", Raleigh (February, 1982); Departments of Chemistry and Biochemistry, LSU (April, 1982); Department of Microbiology, University of Rio de Janeiro, Brazil (June, 1982); International Symposium on Spin Trapping and Nitroxyl Radical Chemistry, Guelph, Ontario, Canada (July, 1981); Southeast Regional Meeting of the American Chemistry Society (November, 1981); and NIH Fogarty Senior Scientist Seminar Series (October, 1981).

Dr. Donald I. McRee: Adjunct Professor, Department of Poultry Science, NCSU; Coordinator USUSSR Coperative Program on Health Effects of Physical Environmental Factors; Organized workshop and hosted Soviet Delegation to U.S.; NIEHS representative on Interdepartmental Radiation Advisory Committee (IRAC) of the National Telecommunication and Information Administration on Biological Effects of Nonionizing Radiation; Representative for DHHS on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines; American National Standards Institute C95 Committee on Safety Standards for Nonionizing Radiation; appointed to National Research Council Committee on Biological Effects of Nonionizing Radiation; Appointed member of IEEE's Committee on Man and Radiation (COMAR).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50005-08 LEB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to increase our understanding of the basic mechanisms of electrolyte movement across the endolymph-perilymph barrier in the guinea pig cochlea under normal conditions and under the influence of physical and chemical agents. The aim of current work is (1) to reveal electrolyte movement (Na^+, K^+ and Cl^-) across the endolymph-perilymph barrier after exposure to intermediate level of noise and (2) to correlate alterations of the ion permeability of the cochlear partition with changes in sensitivity of the auditory organ.

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs anesthetized with pentobarbital sodium were used. The endocochlear potential and Na^+ or Cl^- activity in the perilymph and endolymph were measured in the basal turn of the cochlea with a pair of double barreled ion selective electrodes. The sound-induced responses were also recorded with differential electrodes placed in the basal cochlear turn. Permanent anoxia was induced by injection of lethal dose of pentobarbital sodium. The Na^+ and Cl^- selective electrodes were constructed from neutral carrier Na ion exchanger and Corning 477913 Cl^- liquid exchanger respectively. When active transport is abolished by anoxia, the passive flow of Na^+ across the endolymph-perilymph barrier is

$$J_{Na}^{passive} = \frac{Ve}{A} \frac{d(C_{Na}^e)}{dt}$$

where Ve and A are volume of the cochlear endolymph and the surface area of the endolymph-perilymph barrier respectively. C_{Na}^e represents the Na^+ concentration in the endolymph. Since the Na^+ conductance of the endolymph-perilymph barrier is the ratio of the Na^+ current across the barrier to the electrochemical potential difference for Na^+, the modified Na^+ conductance and Na^+ permeability coefficient of the barrier are

$$G'_{Na} = \frac{\frac{d(C_{Na}^e)}{dt}}{\frac{RT}{F} \ln \frac{(C_{Na}^e)}{(C_{Na}^p)} + \Delta\psi}$$

and

$$P'_{Na} = \frac{RT}{F^2} \cdot \frac{G'_{Na}}{(\overline{N_a})}$$

Using similar calculation to that used for estimate of G'_{Na} and P'_{Na}, the modified Cl^- conductance and permeability coefficient of the endolymph-perilymph barrier were also estimated in normal guinea pigs.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Permeability to Na^+ of the endolymph-perilymph barrier.

1. Normal guinea pigs. The EP recorded before anoxia was 87.7 ± 3.1 mV and the Na^+ activity in the endolymph was 0.77 ± 0.24 mEq/l. Thirty min after anoxia the EP decreased to -30.6 ± 9.2 mV and the Na^+ activity in the endolymph increased to 10.1 ± 2.04 mEq/l. The modified Na^+ conductance and permeability coefficient of the endolymph-perilymph barrier was (2.63 ± 1.44) x 10^{-3} mho cm^{-3} and (18.44 ± 9.33) x 10^{-6} sec^{-1} respectively, when they were averaged from 10 to 30 min after onset of anoxia.

2. Noise-exposed guinea pigs. The Na^+ activity in the endolymph and EP were 0.58 ± 0.16 mEq/l and 87.8 ± 2.8 mV respectively before anoxia was induced. The EP declined to -28.6 ± 4.9 mV and the Na^+ activity increased to 10.14 ± 1.21

mEq/l 30 min after anoxia. The mean values of the modified conductance and permeability coefficient of the endolymph-perilymph barrier averaged from 10 to 30 min after anoxia was (3.19 ± 0.63) x 10^{-3} mho.cm^{-3} and (22.4 ± 4.11) x 10^{-6} sec^{-1} respectively.

3. Kanamycin-treated quinea pigs. In guinea pigs treated with daily doses of 400 mg/kg for 3 to 4 weeks the CM and AP were severely suppressed but the EP was 95.2 ± 2.6 mV and the Na^+ activity in the endolymph was 0.57 ± 0.33 mEq/l. When anoxia was induced, a decrease of EP and an increase of Na^+ activity in the endolymph were much slower than those observed in normal guinea pigs. Thirty min after anoxia the EP was only -6.6 ± 8.3 mV and Na^+ activity was 5.43 ± 1.59 mEq/l. The calculated value of the modified Na^+ conductance of the endolymph-perilymph barrier averaged from 10 to 30 min after anoxia was (2.43 ± 1.03) x 10^{-3} mho cm^{-3} and its permeability coefficient for Na^+ averaged from the same period was (21.54 ± 8.61) x 10^{-6} sec^{-1}.

These data suggest that the permeability to Na^+ of the endolymph-perilymph barrier was not markedly affected by exposure to noise at 115 dBA for 7 days or treatment with kanamycin.

B. Permeability to Cl^- of the endolymph-perilymph barrier.
In normal guinea pigs the preanoxic value of the Cl^- activity was 126.0 ± 1.64 mEq/l in the perilymph and 132.5 ± 1.93 mEq/l in the endolymph. When anoxia was induced, the Cl^- activity in the endolymph decreased and that in the perilymph gradually increased. Thirty min after onset of anoxia the Cl^- activity was 123.0 ± 2.16 mEq/l in the endolymph and 131.9 ± 5.56 mEq/l in the perilymph. The estimated modified conductance for Cl^- of the endolymph-perilymph averaged from 10 to 30 min was (8.40 ± 3.21) x 10^{-3} mho.cm^{-3} and its permeability coefficient was (24.2 ± 9.4) x 10^{-6} sec^{-1}.

The experiments designed to determine the permeability for Na^+ of the endolymph-perilymph barrier were completed. The data on the permeability to Cl^- of the endolymph-perilymph barrier in normal guinea pigs will be used to provide a base line to which Cl^- permeability of the endolymph-perilymph barrier in noise- or drug-treated guinea pigs can be compared.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to various electrolytes has been frequently hypothesized but the supporting data are rather limited. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ears under the influence of physical and chemical agents.

PUBLICATIONS

Salt, A.N., Konishi, T.: Functional importance of sodium and potassium in the guinea pig cochlea studied with amiloride and tetraethyl-ammonium. Jap. J. Physiol (in press).

Konishi, T., Salt, A.N. and Hamrick, P.E.: Effects of exposure to noise on permeability to potassium of the endolymph-perilymph barrier in guinea pigs. Acta Otolaryngol. (in press)

Konishi, T., Salt, A.N. and Kimura, R.S.: Electrophysiological studies of experimentally induced endolymphatic hydrops in guinea pigs. p. 47-58 In: Meniere's Disease ed. by K-H. Vosteen et al. Georg Thieme Verlag. Stuttgart. New York, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-08 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Microwaves on Neural Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Howard Wachtel	Consultant	LEB	University of Colorado

COOPERATING UNITS (if any)
Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Nonionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.4	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Frog sciatic nerves have been exposed to both continuous wave (CW) and pulse microwave radiation. Fatigue or loss of vitality (the ability of the nerve to continue firing under rapid stimulation) have been investigated using microwaves of 2.45 GHz frequency. Distinct changes in the vitality and refractoriness of the exposed nerves were seen in comparison to control nerves for specific absorption rates (SAR's) of 10 mW/g and above. No differences in rundown time were observed between the continuous wave and pulse wave exposure using the same average SAR. No difference in loss of vitality was observed for synchronous microwave pulses with the peak of the compound action potential, quiescent period, or asynchronous pulses which occurred at random periods in relationship to the compound action potentials. Exposures of frog sciatic nerves to sine-wave modulated 2.45 GHz microwaves at frequencies of 16 and 32 Hz are have been completed. A higher average SAR was required to produce a loss of vitality in the exposed nerve then was necessary for CW and pulse microwaves. The loss in vitality was the same for all modulation frequencies.

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the sciatic nerve of the frog, lobster ganglia, abdominal ganglion of the Aplysia, and saphenous nerves of cats will be exposed to CW, pulsed and modulated microwave radiation in the specific absorption rate (SAR) range of 0-100 mW/g. The effects of the radiation on nerve function and the mechanisms of interactions involved in any changes will be investigated.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of isolated frog sciatic nerves to sine-wave modulated 2.45-GHz microwave radiation at SAR's of 10 mW/g and 50 mW/g have been completed using the same waveguide exposure system utilized for CW and pulse-wave exposures. The modulation frequencies were 8, 16, and 32 Hz. Unlike the results of the CW and pulse-wave exposures, we found that at 16 Hz modulation an average SAR of 10 mW/g was not adequate to produce a clear decrement in the vitality of the exposed nerves. The effect was, however, clearly present at an SAR of 50 mW/g and did not appear to be dependent on the modulation frequency. These results suggest that the nerve vitality effect is nonlinear with respect to microwave intensity and thus the modulation envelope is functionally "clipped" below threshold. This in turn necessitates a higher average SAR then would be needed to achieve the same effect with CW or square pulse modulation. This type of nonlinear behavior would be expected if the neural membrane is acting as a diode-like detector of the microwave field.

Additional experiments using sine-wave modulated 2.45 GHz microwave will be carried out in the future. A new waveguide exposure chamber is being constructed which will allow the use of labeled ions and drugs for investigating ionic transport changes through the membranes of nerves. Lobster nerves will be studied to determine their utility for these membrane permeability investigations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The neurological and behavioral effects reported in the literature illustrate that these biological systems are sensitive to microwave radiation. The recommended level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

PUBLICATION

McRee, D.I. and Wachtel, H: Pulse microwave effects on nerve vitality. Radiation Research 91: 212-218, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50017-09 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Microwave Effects on Embryonic Development, Immunology and Fertility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
OTHERS:	James P. Thaxton	Consultant	Poultry Science, NCSU	
	Carmen Parkhurst	Consultant	Poultry Science, NCSU	

COOPERATING UNITS (if any)

Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	.4	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Japanese quail eggs were subjected to 2.45 GHz CW microwave radiation at 5 mW/cm^2 (SAR = 4.03 mWg) during the first 12 days of embryogeny and reared to 22 weeks of age before examination. Humoral immune potential, as indicated by comparable anti-CRBC antibody, IgM and IgG, levels at 0, 4 and 7 days post-immunization in both exposed and control quail, was not affected significantly. However, cell mediated immune potential measured by the reaction to intradermal injection of phytohemagglutinin-P in the wing web, was reduced in the exposed females, but not in the exposed males. Additionally, total leucocyte numbers and absolute circulating numbers of lymphocytes, monocytes and heterophils were increased significantly only in the exposed females. Mice were irradiated at 2.45 GHz for 8 hours per day at a power density of 30 mW/cm^2 during days 1-6 and 6-15 of pregnancy. Samples were obtained on day 17 of pregnancy. The total white blood cell and differential cell counts of peripheral blood samples were not affected by either exposure regimen. No effect was noted on either the erythroid or myeloid mitotic indexes of bone marrow samples.

PROJECT DESCRIPTION

METHODS EMPLOYED: A. The first objective of this project is to determine the effects of 2450 MHz CW microwave radiation on embryonic development of Japanese quail and the subsequent growth, reproduction, biochemistry, immunological response of the maturing quail. The fertilized Japanese quail eggs were exposed to an incident power density of 5 mW/cm^2 (specific absorption rate = 4.03 mW/g) for 24 hours per day for the first twelve days of development. The exposures were carried out in an anechoic chamber, which was environmentally controlled so that the eggs were maintained at optimum incubation conditions (37.5°C and 60% humidity).

B. The second objective of this project is to determine the effects of 2450 MHz CW microwave radiation on the development of rat pups. The parameters examined include growth, biochemistry, hematology, and immunological responsiveness of the maturing rats. Pregnant rats were exposed to an incident power density of 10 mW/cm^2 (specific absorption rate - 2.0 mW/g) for 3 hours per day from day 4 thru 20 of pregnancy. The exposures were carried out in an anechoic chamber which was environmentally controlled.

MAJOR FINDINGS AND PROPOSED COURSE: A. Japanese quail (Coturnix coturnix japonica) eggs were subjected to 2.45 GHz CW microwave radiation at 5 mW/cm^2 (SAR - 4.03 mW/g) during the first 12 days of embryogeny. Following hatching the exposed embryos, as well as non-exposed controls, were reared to 22 weeks of age. Humoral immune potential, as indicated by comparable anti-CRBC antibody, IgM and IgG, levels at 0, 4 and 7 days post-immunization in both exposed and control quail, was not affected significantly. However, cell mediated immune potential measured by the reaction to introdermal injection of phytohemagglutinin-P in the wing web, was reduced in the exposed females, but not in the exposed males. Additionally, total leucocyte numbers and absolute circulating numbers of lymphocytes, monocytes and heterophils were increased significantly only in the exposed females. These data show that exposure of Japanese quail during embryogenesis reduced cell mediated immune potential and induced a general leucocytosis in females.

B. In this study, the influence of 2450 MHz microwave radiation on hematopoiesis in pregnant mice was examined. Dams (CD-1 strain) were exposed to plane wave, 2450 MHz microwave radiation during days 1 through 6 or 6 through 15 of pregnancy. Mice were irradiated for 8 hours per day at a power density of 30 mW/cm^2. Samples were obtained on day 17 of pregnancy. The total white blood cell and differential cell counts of peripheral blood samples were not affected by either exposure regimen. In addition, no effect was noted on either the erythroid or myeloid mitotic indexes of bone marrow samples. Exposure of pregnant mice to microwave radiation under the conditions of these experiments had no overt effect on hematopoiesis.

The proposed course of research is as follows: A. Research will continue using the Japanese quail as a test system. The studies will be expanded to include 6 and 12 week old quail. Longevity studies will also be carried out on the quail exposed *in ovo*. The average life span of Japanese quail is approximately two

years. Normal housing and mating conditions will be maintained throughout the lifetime and reproduction capacity monitored. The ability of adult Japanese quail exposed *in ovo* to respond to thermal stress will be investigated.

B. This project will be completed and extended to studies on the immune capacity of pups from microwave exposed dams.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The question of whether exposure to microwaves during embryonic development has any effect on the development process and function of the organism after maturity must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-07 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Results of behavioral and audiological tests with humans imply that ability to understand speech and other complex signals (particularly in a noisy listening situation) suffers deterioration from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a complex interaction occurs between the various levels of the auditory nervous system and that functionally different receptors of the auditory end organ play a vital role in "sharpening" the sensory process. The objective of this study is to use the rapid signal analysis ability of minicomputers to determine the auditory response to speech and speech-like signals including pseudorandom noise under normal conditions and conditions of auditory fatigue. Auditory fatigue and recovery process of single nerve fibers to speech and speech-like stimuli will be studied. The requirement in high speed data throughput for nearly absolute phase matching of input acoustical and output physiological signals, and for accurately processing neural pulse trains necessitated the design and building of several unique complex peripheral devices.

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to produce speech-like inputs most efficiently, pseudorandom noise which has been amplitude modulated by sinsusuoidal signals is presented to the animal. The pseudorandom noise approximates the constantly changing frequencies of speech while the sinusoidal modulation simulates the varying intensities. When the sinusoidally modulated pseudorandom stimulus is presented to the animal the auditory nerve response is simultaneously recorded in the form of a cycle histogram, the cycle being locked to the period of pseudorandom noise. After summing the histogram many times to ensure statistical validity, cross-properties between the output and the known input, which represent the response characteristic of the peripheral auditory system, can be determined.

1. In order to obtain the temporal characteristics of the input noise a high-speed analog-to-digital converter (ADC) system capable of converting at speeds up to 40-50 kHz was necessary. Three channels (at roughly the same high speed) are needed for this and other planned experiments. Since no commercially available ADC system with a PDP-11 interface with sufficiently high speed existed, it was necessary to design and construct one around commercially available high-speed ADC modules and interfacing boards. This project was a cooperative venture involving Computer Engineering, BB, our electronic consultant, and LEB personnel.

2. For acquiring the histogram data, a special high-speed clocked pulse counter interfaced directly with the computer was designed and built on a commercially available interface board by LEB personnel with design guidance from Computer Engineering, BB. As with the ADC, the special design was primarily necessitated by the 40-50 kHz data rate.

3. As an intrinsic adjunct to any high-speed ADC system, a low-pass sharp-cutoff filtering system must be provided in order to prevent aliasing of high frequencies. Phase matching requirements of such filtering systems when time dependent cross properties are to be measured far exceed those normally available filters. State of the art elliptic filters providing a higher maximum data rate and increased phase accuracy became available last year and were built into the system by LEB personnel.

4. Since the binary pseudorandom noise conventionally used to test auditory system function was recently shown to generate 2nd order distortion of the same type as the auditory system, a computer software based system for generating trinary pseudorandom noise was developed by LEB personnel in conjunction with an expert (consultant) on digital filtering.

5. An electronic (hardware) auditory nerve simulator (analog in, digital pulse train out) was developed in order to facilitate shakedown and evaluation of various strategies for computerized acquisition of nerve fiber tuning curves. This system is much more efficient one than use of live animal preparations.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year software and hardware development was extended to allow probe tube microphone-external ear cavity spectral transfer function and single nerve fiber tuning curves to be efficiently acquired from live anesthesized guinea pigs.

Hardware development is 100% complete and initial software development is about 95% complete. Because the project involves a major commitment of resources, it is difficult to say exactly when this series of experiments will begin on a full time basis.

A general purpose transfer function analysis program accepting analog inputs while outputting transfer function magnitude and phase, coherence, input and output power spectra, auto and cross correlation and impulse or step response is in place and operating as designed. While the laboratory is still working on a sound transducer whose distortion levels are desirably low, pilot experiments will be possible.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years, it has become clear that the ability of the cochlear transducer to respond to pure tones is not an adequate indicator of its ability to respond to the natural complex stimuli present in the real world. Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistent in spite of the fact that complex stimuli perceived in a noisey environment constitute the natural situation. We hope to obtain data which will begin to fill this void. An increased insight into how complex signal analysis in the auditory system is impaired in noise-induced hearing loss could lead to development of more suitable hearing aids.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-06 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Optical Fiber Motion Detector Systems for Auditory System Measures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer LEB NIEHS
OTHERS:	William Henson	Professor of Anatomy, UNC School of Medicine Chapel Hill, N.C.
	Thomas Hodgson	Director, Center for Acoustic Studies NCSU

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Only a few techniques are capable of the resolution and frequency response necessary for auditory system motion measures; i.e., 10^{-2} - 10^{2} Angstroms across 100 Hz-20 kHz bandwidth. Previous theoretical and experimental efforts have led to determination of intrinsic performance limits and identification of the parameters relevant to optimization for given objectives. These analyses showed optic lever systems to be competitive with interferometric and optical heterodyneing techniques and better than capacitive probe techniques in resolution and an order of magnitude simpler and less expensive than either. Continuing refinement and explorative efforts have been focused in three areas: experimental determination of the optimum probe size/linear range trade off and development of automatic in site calibration circuitry for umbo/ossicular chain measures; development and testing of high speed differentiation circuitry allowing correlation of mechanical impulses with acoustic emissions from impacted plate and other radiating structures and work on specialized probes for measurement of basilar membrane vibration. The latter employ fiber optic couplers which allow a single fiber to act as both sender and receiver.

PROJECT DESCRIPTION

METHODS: Physics, electro optics, optics (fibers), electronic engineering, computer modeling, and noise measurements.

MAJOR FINDINGS AND PROPOSED COURSE: Intrinsic performance limits of this non-contact displacement measuring technology have been quantified, experimentally verified, and published in a form which facilitates design optimization for a variety of applications. Further performance improvements have been focused on more efficient illumination sources and rejection of the 60 Hz harmonic room lighting since other components have been pushed to theoretical limits. It was also determined that by optimizing electronic differentiator parameters and utilizing recently developed very high speed op amps, the displacement signal had sufficient dynamic range to give high dynamic range velocity and acceleration values. Measures of velocity and acceleration obtained from impacted plates obtained by conventional accelerometers were compared with values obtained by this technology in an experiment be signed to correlate shock and sound pressure level amplitude and waveform. A paper which details this additional capability was published, and others have obtained grants based on using this development as the vibration sensing element of an acoustic intensity meter.

Auditory System Optimization: The small size of the termination of the ossicular chain (umbo) in the center of the tympanic membrane coupled with the relatively large "DC" movements imposed on the ossiclar chain by the middle ear muscles pose serious motion measurement problems. Since working distance and linear range are directly proportional to fiber diameter, use of fibers which are too small allows the spontaneous "DC" motions to carry the umbo beyond the linear range of the lever, while use of oversize fibers results in illumination of areas bigger than the umbo, introducing measurement errors. After analytical determination that the optimum fiber diameter was on the order of 125 microns, fibers of 100, 125, and 200 microns diameter were ordered and probes fabricated. Displacement detection limits on the order of 1/10 to 1/100 Angstrom per Hertz bandwidth were obtained from the ossicular chain utilizing mylar reflectors, and of 1 Ångstrom per Hertz bandwidth without reflectors.

Fiber Lever Optimized for Basilar Membrane Vibrations: Single fiber tip probes (utilizing fiber coupler technology) having the necessary size (50 μ) and resolution (1A/$\sqrt{Hz}$) necessary for basilar membrane measures have been fabricated and problem of making light turn 90° (in less than 100 μ) inside the cochlea also appears to have been successfully solved. Design and testing of electronic circrutry meeting goals of 25 kHz bandwidth which keeping the noise floor at photo current generated shot noise levels has been accomplished. Pilot tests utilizing preserved (non live) bat and guinea pig cochleas are in progress and a series of experiments utilizing live animals are planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Shock and vibration transducer: Reduction of harmful noise emissions from machinery and other rotating sources can best be avoided at the design stage provided theory on which predictions of the noise emissions resulting from mechanical shock is available.

The use of noncontacting fiber levers is desirable and high frequencies are important. Others are attempting to construct acoustic intensity meters based on this development. Such meters would contribute significantly to identification of noise emission sources within complex systems, including industrial machinery.

Optimized Ossicular Chain and Basilar Membrane Auditory Motion Transducer: A convenient means for making audio frequency displacement measures of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL) has long been needed in the study of auditory distortion and hearing loss mechanisms. Reliable measures of the motion of the basilar membrane would resolve present uncertainties concerning non-linear plateau effects at high intensities and frequency resolution from high and low frequency slopes of the maximas; these uncertainties block confirmation of hearing theories at the intracochlear mechanical level. Despite the fundamental and theoretical importance of such information attempts to obtain it have been largely frustrated because of the technical difficulties involved.

In addition to these specific applications, optic levers have been utilized to measure the dynamic force exerted by muscle myofibrils, as the basis for ultra-sensitive pressure transducers for cardiovascular and other clinical purposes. Although the non-contacting characteristic of optic levers uniquely quailfy them for biological (and many mechanical) applications, application of the technique has previously suffered from lack of a theoretical basis from which design optimization could proceed, and from the relatively short working distances and low detection limits associated with the commerically available models. Since the theoretical relationships have been developed for both laser and Lambertian sources and confirmed by measurement, it is now possible to predict accurately the ultimate resolution for given fibers, bundle configurations and illumination sources. Illumination sources and fiber devices made commerically available within the past year by telecommunications industry suppliers have significantly increased device resolution.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50022-06 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Electronic and Electro Acoustic Devices for Hearing Loss Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Reginald O. Cook Acoustical Engineer LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) The purpose of this project is to refine a pneumatically powered impact noise generator whose peak level, rise time, and decay can be experimentally manipulated. Mechanical, acoustic and reliability parameters are being measured currently in preparation for (animal) exposure experiments.
2) A closed system electroacoustic transducer having the ability to deliver high level, wide band minimum distortion sounds to the eardrum of guinea pigs has long been needed. Purpose to develop such a device.
3) Purpose is to develop a simple instrument with which significant speech discrimination test parameters can be manipulated. These parameters include peak level, long-term equivalent level, defined pink noise and speech spectrum noise for masking purposes and a means for modulating both.
4) Design of the necessary electro optics for resolution of 10^{-9} watts (DC) of 8200Å illumination returned from the vibrating lasilar membrane. Design requirements were 25 kHz BW and noise at or below inescapable photo current generated shot noise levels, and approximately 1Å/$\sqrt{Hz}$ resolution.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: 1) Mechanical, electronic, and acoustical engineering concepts were utilized in the design phase, which had been preceded by analytical and experimental investigations of the acoustic characteristics of mechanically generated impacts implicated in hearing losses associated with human exposures.

2) Electroacoustic concepts will be employed in the design of these devices.

3) Electronic design utilizing state of the art electronic devices were employed to assure the desired auditory characteristics.

4) Analog and digital electronic engineering concepts were used to design the simulator.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The pneumatic drive mechanism under test appears to have solved the failure rate problem associated with earlier solenoid operated device. Previous tests with animals indicated physiological parameters associated with hearing impairment (CM, AP) deteriorated in different ways when exposed to equal energy impact vs. continuous noise. Chronic impact exposure experiments will begin soon. These were delayed due to factors beyond our control, namely installation of adequate compressor.

2) A manufacturer of high frequency audiology instrumentation is presently working with us on development of this device.

3) The 2nd generation model has been constructed and is currently being evaluated.

4) "State of the art" devices have been constructed which achieve about 90% of design goals. A method for sputtering gold on the fiber tip to facilitate reflection has been perfected and the highest intensity (200 watts/$_{cm}$2) non laser optic source available has been procured.

SIGNIFICANCE TO BIOLOGICAL RESEARCH AND PROGRAMS OF THE INSTITUTE: 1) The fact that it is not possible to reproduce impact sounds using conventional audio equipment has resulted in few, if any, well controlled laboratory studies of hearing damage from a type of noise which pervades industry and society. There is strong suggestive evidence that high intensity, short rise time peaks, rather than steady state levels, are the major contributor to hearing loss. Because the temporal summing characteristics of the human auditory system operate over a longer time span than the duration of impact sounds, perceived loudness is not proportional to peak level or to peripheral organ damage possibilities. This effort should make refined studies of hearing loss/impact noise parameters possible.

2) A closed system electroacoustic transducer having the ability to deliver high level, wide band, minimum distortion sounds to the eardrum of guinea pigs has long been needed. Sound sources with which precise control. It is believed that current electroacoustic transducer technology can be exploited to achieve the needed advances.

4) Current practices in testing of speech materials for the hearing impaired have either relied on peak reading instruments, which bias in favor of more distorted speech, or on expensive computers beyond the reach of many audiology labs or clinics. A simple, inexpensive device will make it possible for many more labs to do competent speech discrimination testing. Speech discrimination is used in comparing initially identical speech after passage through different hearing aids, or other fidelity degrading communication systems.

4) Most researchers who would like to try to measure basilar membrane motion have been frustrated by the highly complex signal processing and stability requirements of the laser interferometic process generally thought necessary. The amplitude modulated technique we have developed avoids many of noise problems at little loss in detection limit, and by constraining the light beams amplitude inside optic fibers, interference is minimized and stability maximized.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50028-04 LEB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effects of Noise and Drugs on the Electrochemistry of the Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Determination of electrochemical gradients across the cochlear hair cell membranes is essential for understanding the biophysical basis of cochlear transduction. The aim of this study is to measure the electrochemical driving force for the movement of K^+, Na^+ and Cl^- in the cochlear hair cells in normal and noise-exposed guinea pigs.

PROJECT DESCRIPTION

METHODS EMPLOYED: The measurement of the electrical potential and the potentiometric determination of K^+ activity difference across the cell membrane were accomplished with double barreled K^+ selective liquid membrane electrodes. Judging from an electrode resistance of 20-50 megohms when the blanks were filled with 3M-KCl solution, the tip diameter of each barrel was less than 1 μm. The mean slope of K^+-selective electrode was 37 mV. The mean selectivity coefficient over Na^+ was 0.025. Exploration of the organ of Corti with K^+-selective electrodes was carried out in guinea pigs anesthetized with pentobarbital sodium. After removal of the round window membrane a K^+-selective electrode was advanced toward the organ of Corti by means of a piezo-electric microdriver. A 3M-KCl agar Ag/AgCl electrode was placed in the intact neck muscles and used as a reference. During penetration of the organ of Corti with a K^+-selective electrode, both electrical potential and K^+ potential were recorded on a strip chart recorder. The cochlear microphonics were also recorded from the potential barrel and photographed on a running film. The criteria for a successful impalement of cell membranes were a) an abrupt appearance of a negative dc potential and an associated increase of K^+ activity, b) maintenance of the plateau value of the dc potential and K^+ activity with no more than 10% variation for at least 5 sec and c) return of the dc potential to the original level when the electrode was withdrawn to the perilymph. Successful puncture of hair cell membranes, in addition to the above criteria, was accompanied by a sudden increase in the ac component of the receptor potential and a return of CM magnitude to the original level when the electrode was withdrawn. A group of guinea pigs was exposed to broad band noise at 115 dBA for 7 to 10 days. A second group of guinea pigs was kept in a quiet environment.

MAJOR FINDINGS AND PROPOSED COURSE: Data were collected from 47 successful cell punctures in normal and 38 in noise exposed guinea pigs. Out of these cells 9 cells in normal and 8 cells in noise exposed guinea pigs could be categorized as hair cells.

The K^+ activity in the extracellular fluid in the organ of Corti was 1.98 ± 1.14 mEq/l in normal animals and 1.93 ± 0.86 mEq/l in noise exposed guinea pigs. The intracellular K^+ activity of hair cells was 64.8 ± 43.6 mEq/l in normal guinea pigs and 66.5 ± 36.8 mEq/l in surviving hair cells in noise exposed guinea pigs. The resting potential was -82.4 ± 18.0 mV in normal and -64.3 ± 22.1 mV in noise exposed hair cells. The electrochemical potential difference for K^+ across the basolateral hair-cell membrane was 6.9 ± 21.5 mV in normal and 28.6 ± 22.0 mV in surviving hair cells in noise-exposed guinea pigs. These indicate that in both normal and noise exposed hair cells the K^+ ions are nearly in electrochemical equilibrium distribution across the basolateral cell-membrane. The K^+ activity of the endolymph was 113.8 ± 6.7 mEq/l and 129.6 ± 8.4 mEq/l in normal and noise exposed quinea pigs respectively. The endocochlear potential was 84.7 ± 4.2 mV in normal and 80.5 ± 5.1 mV in noise-exposed guinea pigs. Thus the electrochemical gradient for K^+ across the apical hair-cell membrane was 196.4 ± 20.8 mV in normal and 195.3 ± 15.8 mV in noise-exposed but surviving hair cells. The CM in the scala tympani and scala media was severely suppressed in noise exposed guinea pigs. The ratio of intracellular to extracellular CM was greater in noise exposed hair cells than in normal hair cells.

The experiments to determine the electrochemical profile for K^+ across the hair-cell membranes have been completed and a manuscript is in preparation for publication. We plan to determine the electrochemical profile for Na^+ and Cl^- across the hair-cell membrane.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Determination of the electrochemical profile for K^+ across the hair cell membrane is important for the understanding of the cochlear transduction. The present study will make a significant contribution to understanding the physiology of noise induced ear damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50031-05 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Mast Cell Degranulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHER:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The membranes of both purified mast cells and mastocytoma cells have been studied using spin-labeled probes. The membrane fluidity of the neoplastic cells was significantly greater than that of the normal mast cells. The character of the spin probe was also found to be important in determining the accuracy of fluidity studies. Highly charged probes (such as fatty acids) may report a lower apparent fluidity due to the anchoring influence of their charged end group. Compound 48/80, a mast cell degranulating agent, had no effect on any of the lipid spin labels studied; however it was able to reverse the effect of magnetic interactions between closely adjacent spin labels. This suggests that 48/80 may increase the available membrane binding sites for the spin labels. Compound 48/80 also affected the state of MSL-ghost proteins by causing an increase in the population of highly immobilized label. The effect of trypsin was similar between MSL-ghosts and SL-48/80-labeled mast cells. Fluorescence microscopy has shown that mast cells and mastocytoma cells bind R-48/80 on the plasma membrane at low concentration and at high concentration, the binding also occurred at anionic sites in the cell interior.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat mast cells were isolated according to well established procedures and purified by centrifugal elutriation. The mastocytoma cells were an established cell line. The effect of 48/80 on the environment of 5-doxyl-stearic acid (5-DSA) and 5-doxylmethylstearate (5-DMS) was determined using the method in which the half width of the high field hyperfine extrema was measured at half its height. Rotational correlation times and order parameters were also calculated for the spin-labeled fatty acids. Fluorescence microscopy was carried out with a Leitz Dialux 20 microscope equipped with pleopak incident light fluorescence illuminator.

MAJOR FINDINGS AND PROPOSED COURSE: The rat peritoneal mast cell has been widely studied because of its role in the anaphylactic response and because it is a useful model system for examining the secretory process. We have studied the membranes of rat mast cells and peritoneal and pleural cells and also murine mastocytoma cells using the spin label method and fluorescence microscopy. We have also studied the effect of compound 48/80, a highly potent and efficacious stimulator of mast cell secretory action. For our electron spin resonance (ESR) studies, large numbers of purified mast cells were needed. We have therefore employed the technique of centrifugal elutriation to provide rapid purification of large numbers of cells. Human erythrocyte ghost membranes covalently labeled with a maleimide spin label have also been used to study the interaction of 48/80 with the membrane proteins.

The effect of 48/80 concentration on cell binding was determined by fluorescence microscopy. Rhodamine labeled 48/80 (R-48/80) was studied at various concentrations using both mast cells and mastocytoma cells. Using this technique we were able to observe and photograph the binding of 48/80 to various cell types.

Rat peritoneal mast cells, which constitute 6-10% of the peritoneal and pleural cell population, can be successfully and rapidly purified using centrifugal elutriation. It was necessary to include serum albumin for the cells to retain their ability to secrete histamine. Elutriated mast cells released histamine in a manner similar to cells stored in the presence of albumin.

The ESR spectra of 5 DMS in both mast cells and mastocytoma cells suggested that the label was more highly immobilized in the mast cells than in the mastocytoma cells. In contrast, 5 DSA was very highly immobilized in both mast cells and mastocytoma cells and there was no apparent difference between the two cell types. The motion of the nitroxide group attached to stearic acid was very dependent on the position of the nitroxide with regard to the charged end of the molecule. There was a progressive increase in the motion of the nitroxide as it was located further from the ionized carboxyl group and, presumably further into the hydrophobic region of the cell. The signals from the spin labeled methyl stearates show a similar pattern, however, their motion was not as strongly influenced by the position of the nitroxide. In mastocytoma cells, all of the labels except the 5 DSA were less immobilized than in the mast cells. Also, in each case the nitroxide attached to the acid was more highly immobilized than its counterpart attached to the ester. However, this difference decreased as the nitroxide group was moved further from the charged end of the molecule.

The correlation times derived from spin labels in mast cell depleted leukocytes indicate that mast cell membranes are similar in fluidity to the other assorted white blood cells found in the rat peritoneal cavity.

Our data show that the apparent membrane fluidity increases as the reporter group is located further into the hydrocarbon region and further away from the charged end of the molecule. This finding is consistent with the current theories regarding membrane structure in that the most molecular ordering occurs at the surface and interface regions, presumably due to the presence of peripheral proteins and the glycerol backbone of phospholipids. In both mast cells and mastocytoma cells, the molecular ordering as reported by the 5 DSA was similar. However, the rotational correlation time (and presumably the membrane fluidity) increases very rapidly in the mastocytoma cells as reported by the signal from the 12th carbon, and there is little change from the 12th to the 16th carbon. In the mast cell, the increase in fluidity was more gradual and the abrupt change came between the 12th and the 16th carbon atoms. This effect was more pronounced with the acid derivatives, although the ester derivatives also showed a similar pattern. This finding indicates a fundamental difference between the mast cell and the mastocytoma cell membrane; however its significance if any, in explaining the neoplastic character of the mastocytoma cells, remains unclear.

The difference in motion seen between the acid and ester derivatives was greatest when the nitroxide was placed nearest the acid or ester group (5-DMS, 5 DSA), and diminished as the nitroxide was situated further away (16 DMS, 16 DSA). This was seen with both mast cells and mastocytoma cells and is probably due to the stronger "anchoring" effect of the acid moiety. The charge on the stearic acid therefore provides an additional constraint on the motion, and consequently, the apparent membrane fluidity is lower when the reporter group is situated close to its influence. If it can be assumed that the acid and the ester have the same orientation within the membrane, then the stearic acid probes were reporting artificially low fluidities for both the 5-nitroxide and the 12-nitroxide derivatives.

The current study shows that the mastocytoma cells are probably more fluid in all regions of the membrane. The 5 DSA was the only probe to report a similar fluidity for both cell types, however this was probably due to the strong influence of the charged group anchoring the molecule rather than the actual condition of the membrane. The 5 DMS probe suggests that mast cell membranes are indeed more rigid at the interface and this difference persists into the hydrophobic region of the membrane. This study shows that the use of 5-doxyl stearic acid to compare different membrane types can lead to erroneous results and that several different probes should also be included in a complete study of membrane fluidity.

There was no effect of 48/80 on stearic acid and ester spin labels incorporated into either mast cells or mastocytoma cells; and the apparent molecular motion of the spin labels was not changed even at 48/80 concentrations far exceeding that which was maximally active. The effect of spin label concentration in the membrane has been studied. When the cells were incubated with spin label concentations higher than 2.5×10^{-3} M, the extrema of the labeled cells were broadened, presumably due to magnetic interactions between closely adjacent nitroxide

molecules. The data showed that the effect of 48/80 on the spectra was only apparent in cases where there were magnetic interactions present. Compound 48/80 reduced the magnetic interactions and the spectra resumed a normal shape. There was no effect of 48/80 on cells in which the membrane concentration of label was magnetically dilute.

Although compound 48/80 precipitates a major molecular rearrangement in mast cell membranes, it was not possible to demonstrate any effect of this agent on bound stearic acid and ester probes which were magnetically dilute. Therefore, the absence of any effect by 48/80 on the labels suggests strongly that its action does not result in a permanent major fluidity change among the membrane lipids or at the interface.

The effect of 48/80 on high concentrations of the stearate spin-label in the mast cell membrane indicates that 48/80 increased the distance between interacting spin-labeled molecules, thereby reducing intermolecular magnetic interactions. One possible explanation for this observation is that compound 48/80 increases the number of binding sites for the spin labels. We have previously suggested that 48/80 may act by causing a partial denaturation of the membrane proteins. If this mechanism is correct, the denatured membrane proteins may provide the additional binding sites which are generated by 48/80.

Spin-Labeled Ghosts: We have spin-labeled ghost membranes using a maleimide derivative (MSL) which covalently binds to membrane proteins via their sulfhydryl groups. We have found that 48/80 causes an increase in the population of more highly immobilized spin labels. These results are direct evidence of a membrane protein interaction by 48/80. We have also found that treatment of MSL-ghosts with trypsin causes rapid destruction of the highly immobilized sites. Similar treatment of purified mast cells labeled with spin-labeled 48/80 yields the same result. These data indicate that 48/80 binds to a general site on cell membranes which may be similar to that of the MSL and is probably a protein.

Fluorescence Microscopy: Compound 48/80 was covalently labeled with the fluorescent moiety, rhodamine. At low concentrations rhodamine 48/80 (R-48/80) binds to the plasma membrane of both mast cells and mastocytoma cells. In some cases we have observed "patching" of the fluorescence intensity which would indicate aggregation of the receptor sites. At high concentration, R-48/80 bound to anionic intracellular structures. The R-48/80 apparently increases the membrane permeability, allowing access to the cell's interior. In mast cells, R-48/80 binds to the basophilic granules, whereas in mastocytoma cells it binds to the nucleus.

We have made a detailed study of mast cell and mastocytoma cell membranes in order to understand better the underlying mechanisms behind non-cytotoxic histamine secretion. We have found significant differences between their membrane fluidities. We have also shown that mast cell membranes are similar in fluidity to that of the other normal cells found in the peritoneal and pleural cavities and that this fluidity is unaffected by 48/80. This indicates that the ability to secrete histamine is unrelated to membrane fluidity. In addition we have shown by fluorescence microscopy that 48/80 binds to the outer membrane at low concentrations and to inner constituents at higher levels.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunological stimulation of histamine secretion via IgE antibody-antigen complex formation mediates the symptoms of asthma, hay fever and anaphylactic shock. In addition to this reaginic response, histamine secretion can also be initiated by several drugs and chemicals. Morphine, curare, chlorpromazine, concanavalin A, dextran, calcium ionophores and 48/80 are among the many agents in this category. Histamine secretion by non-immunological means, therefore, presents a serious health hazard to those who are exposed to such drugs for therapeutic reasons. Mast cells are also implicated in the inflammatory reactions associated with photoallergic and phototoxic dermatitis. In addition, they may also exacerbate the conjunctivitis and pulmonary distress caused by certain xenobiotics in the atmosphere. With the aid of biophysical techniques and compound 48/80, we are studying the molecular mechanism of histamine secretion. These studies may lead to the development of a safe method of control over both immunologically and environmentally related adverse conditions due to histamine secretion by mast cells.

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Ortner, M.J. and Chignell, C.F.: Spectroscopic studies of rat mast cells, mouse mastocytoma cells and compound 48/80. III. Evidence for a protein binding site for compound 48/80. Biochem. Pharmcol. 30: 1587-1594, 1981.

Ortner, M.J. and Chignell, C.F.: The effects of concentration on the binding of compound 48/80 to rat mast cells: a fluorescence microscopy study. Immunopharmacology 3: 187-191, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50032-05 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Binding of Copper to Human Serum Albumin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Mohanakrishnan	Visiting Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The binding of copper (Cu^{2+}) and nickel (Ni^{2+}) ions to serum albumin has been studied by ^{35}Cl nuclear magnetic resonance (NMR). The number of high affinity binding sites estimated by the NMR technique agreed well with previous data obtained with an ion-specific electrode. The estimated correlation time of albumin bound Ni^{2+} suggests that the N-terminal metal ion binding site undergoes more rapid motion than the protein molecule as a whole. Circular dichroism studies showed marked differences in Cu^{2+} and Ni^{2+} binding to dog serum albumin as compared to human and bovine serum albumin. Attempts to use ^{19}F NMR to monitor ligand binding to human serum albumin were unsuccessful.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fluorescence and circular dichroism techniques were used to study the binding of small molecules to human serum albumin. Specifically labeled HSA was synthesized. Proton, carbon-13 and fluorine-19 spectra of HSA and specifically labeled HSA were observed.

MAJOR FINDINGS AND PROPOSED COURSE: The binding of Cu^{2+} and Ni^{2+} to bovine, human and dog serum albumins was studied by ^{35}Cl NMR probe technique. The study indicates two different types of binding sites. For nickel, the number of relatively "high" affinity sites estimated are 1 to 2 for human, 2-3 for bovine and above 6 for dog serum albumin. The estimated rotational correlation time for chloride ions at the metal site was of the order of one picosecond. There was good agreement for the number of "high" affinity Ni^{2+} and Cu^{2+} sites for dog serum albumin. The initial irregular change of line width made it impossible for a similar comparison for the other two albumins. The estimated correlation times probably reflects a faster motion at the N-terminal site rather than albumin as a whole. The number of Ni^{2+} sites found from ^{35}Cl probe studies are in reasonable agreement with the number of Cu^{2+} sites found using ion selective electrode.

Cu^{2+} and Ni^{2+} binding to dog serum albumin was studied by circular dichroism. In the case of Cu^{2+}, the CD bands observed below 600 nm for bovine and human serum albumins were absent for dog serum albumin. Unlike, bovine and human albumins, nickel carbonate (in carbonate buffer pH 10.3) precipitated with dog serum albumin. The tendency for chelation is of the order BSA $\sim$ HSA>CO_3^{2-}> dog serum albumin. These results are in agreement with other previous studies.

The lone sulfhydryl group of HSA was labeled with dansyl aminoethyl and iodo-acetyl aminoethyl amino napthalene sulfonyl groups. The labeled protein was studied using fluorescence and induced dichroism. The fluorescence of the bound label was quenched more than 50% by the first equivalent of bilirubin and to about 10% by the second equivalent. Lifetime measurements will be carried out with the labeled protein in the presence and absence of small molecules. Critical distance measurements will be carried out from the label to the lone trp and fatty acid and bilirubin binding sites. The induced dichroism was found to be affected to a small amount by an initial addition of fatty acids. From the fatty acid binding studies, the dansyl probe seems to be in a more chiral environment than the ANS-type probe used.

HSA was labeled at the aspirin acetylation site with 2,6-dinitro-4-trifluoromethyl benzene sulfonyl group and ^{19}FNMR spectrum of the labeled protein was observed. Studies with fluorosalicylic acid and diflunisal met with little success because of the instrumental limitations in partial proton decoupling.

A tetrapeptide, Asp-Ala-His-Lys-OMe, with the sequence same as in N-terminal copper site was synthesized. Currently, experiments are being carried out to characterize the tetrapeptide and to study the binding of Cu^{2+} and Ni^{2+} as a model to N-terminal Cu^{2+} site. Proton NMR spectra of HSA at 400 MHz show more than 12 C_2 and C_4-H protons of the His residues. No further studies will be carried out on this project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Copper and nickel are implicated in several pathological and toxicological conditions. HSA is the major carrier of metal ions and small molecules in the serum. It is hence of tremendous significance in studying HSA and its metal ion and drug binding to unravel the underlying biological processes/mechanisms.

PUBLICATION

Mohanakrishnan, P. and Chignell, C.F.: Ion-specific electrode study of copper binding to serum albumins. J. Pharm. Sci. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50038-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwave Radiation on the Cardiovascular System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Melvyn Lieberman	Consultant	LEB	Duke University

COOPERATING UNITS (if any)

Physiology Department, Duke University

LAB/BRANCH
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SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine the influence of microwave radiation on cardiac tissue in vitro and in vivo. A method for exposing isolated rat atria to microwave radiation has been developed. The data suggest that 2.45 GHZ CW microwave radiation of 2 or 10 mW/g has no overt effect on the rate or force of contraction of isolated atria. The influence of microwave radiation on the response of isolated atria to drugs are being undertaken. Also, certain biochemical and physiological parameters, which are indicative of cardiac function, have been measured in unanesthetized rats during whole body ventral exposure to 2450 MHz CW microwaves. Preliminary data suggest microwave exposure of 10 mW/cm^2 for 4 hr has no effect on heart rate, mean arterial blood pressure or colonic temperature.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: a. Isolated rat atria maintained at either 22° or 37°C were exposed to microwave radiation at specific absorption rates of 2, 10 and 100 mW/g for periods up to 1.5 hours. During exposure, contraction rate and contractile force were monitored. For each experiment 2 pairs of atria were used, one control and one exposed, which were placed in specially designed tubes located in a waveguide exposure apparatus. In addition, the response of the tissue to drugs has been determined during microwave exposure. The drugs used included propranolol, isoproterenol and verapamil.

b. Adult male rats were exposed to whole body microwave radiation of 2 and 10 mW/cm^2 at carefully controlled temperatures and exposure levels. Using a specially designed irradiation chamber, rats were exposed either dorsally or ventrally, and certain hemodynamic (blood pressure, heart rate, hematopoietic and biochemical parameters were measured during 4 hour microwave exposure. The temperature humidity and noise level in the exposure chamber were maintained at 23°C, 60% and 70 dB respectively during the experimental period.

MAJOR FINDINGS AND PROPOSED COURSE: a. The data indicate that the exposure rates used (2 and 10 mw/g) has no overt effect on the rate or force of contraction of isolated atria at either incubation temperature. At 22°C the rate of contraction was 120 beats per minutes for both the control and exposed atria. Atria maintained at 37°C had a rate of contraction of 215 beats per minute, and was also unchanged by microwave exposure. These experiments have been extended to examining the response of atria to drugs during microwave exposure.

b. The data given for this experiment is the result of preliminary experiments and may be different from the final results obtained at the completion of the study. In the sham exposed rats the mean aterial blood pressure (MABP), heart rate (HR) and colonic temperature were 120 $\pm$ 10 mmHg, 300 $\pm$ 30 BPM, and 37.5 $\pm$ 0.8°C respectively. Four hour exposure to 10 mW/cm^2 microwave radiation had no influence on these parameters. In addition, a number of hematologic parameters were examined during the exposure period. No differences between the sham (0 mW/cm^2) or exposed (10 mW/cm^2) groups were noted for white and red cell numbers hematocrit, or plasma protein. This study will be complicated in the coming year. The next study will examine the influence of microwave radiation on the biochemical and cardiovascular sequelae of endotoxin shock. Preliminary data suggest low level microwave radiation may increase the resistance of rats to endotoxin.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. This project is designed to provide a comprehensive and integrated study of the possible effects of microwave radiation on the cardiovascular system. By using *in vivo* and *in vitro* techniques microwave interactions with cardiovascular system can be evaluated more effectively. This research on the effects of microwaves on the cardiovascular system is directed toward the mission of NIEHS to determine the health effects of physical factors in the environment.

PUBLICATION

Galvin, M.J., M.S. Dutton, D.I. McRee. Influence of 2.45 GHz microwave radiation on spontaneously beating rat atria. Bioelectromagnetics (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50039-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effect of 2450 MHz Microwave Radiation on Mast Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation Workgroups

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of microwave radiation was studied on active secretory cells. Rat peritoneal mast cells were exposed to 2450 MHz microwave radiation at specific absorption rates (SAR) of 8.5 and 42.5 mW/ml for periods of up to 3 hrs. Cells were maintained throughout exposure at 37°C. There was no effect on cell viability or spontaneous histamine release. Mast cells exposed to compound 48/80 after prior irradiation or during simultaneous irradiation secreted histamine in a manner similar to unexposed cells. In addition, mast cells exposed to concanavalin A or A23187 during simultaneous irradiation secreted histamine in a manner similar to unexposed cells. These studies are being extended to determine the influence of whole body exposure of rats to microwaves or mast cell physiology.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat peritoneal mast cells were obtained by peritoneal cavity lavage. The cells were irradiated in a waveguide exposure system using a frequency of 2450 MHz. Dosimetry in the cell chamber was determined directly from the time-temperature profiles to be 8.5 and 42.5 mW/ml respectively. The temperature during exposure was maintained at 37°C using a circulating water bath. Control cells were incubated 9.5 cm away from the microwave source and were not exposed to the radiation. After irradiation, samples of cells were removed and added to either Lockes solution or compound 48/80. In addition, cells were treated with concanavalin A, and A23187 during microwave exposure.
The drug-induced histamine release was terminated by addition of antimycin to the cell suspensions.

MAJOR FINDINGS: Mast cells were irradiated in a wave guide microwave exposure chamber at 2450 MHz with power absorptions of 8.2 and 41.0 mW/g for periods up to 3 hours. These levels of microwave absorption caused no change in the morphological characteristics or viability of the cells. Irradiated mast cells were stimulated with compound 48/80, a potent, non-cytotoxic histamine releasing agent. The dose response curves showed that neither prior nor simultaneous irradiation of mast cells at 37°C affected 48/80 induced secretion. When irradiated mast cells were treated with Con A (25 or 100 µg/ml) or A23187 (0.25 or 1.0 µM), neither of the microwave dosages influenced the release of histamine. These data suggest that microwave radiation at the frequency and absorption rates used in these studies has no influence on histamine secretion evoked by 48/80, calcium influx of lectin binding with cells are maintained at 37°C.

Mast cells irradiated at 44.0°C responded to 48/80 in a manner similar to identical cells heated conventionally to 44.4°C. In contrast, cells irradiated at 43.5°C. were unchanged from cells heated conventionally to 43.5°C. This indicates that the SAR used (41.0mW/ml) did not heat the cells more than 0.9°C above ambient levels, and that the irradiation procedure may have heated the cells 0.4°C above ambient.

These studies will be extended to examine the influence of whole body microwave exposure on peritoneal mast cells. In addition, rats which have been depleted of mast cells by 48/80 injections will be exposed to microwaves during mast cell regeneration. These two additional studies will provide needed information on the interaction of microwaves with cells and body systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough study of the effects of microwave radiation on all biological levels must be completed before microwave radiation exposure can be considered safe. Waveguide systems have been developed to study the effects of nonionizing radiation on cell membranes. The complicated series of events resulting in mast cell membrane fusion and histamine secretion are unimpaired by the dosage and frequency of microwave radiation used in these experiments. Studies are being planned for assessing the effects of whole body microwave irradiation on mast cell function in rats. By using in vivo and in vitro techniques microwave interaction with cells can be emulated more effectively. This research on the effects of microwaves on the cardiovascular system is directed toward the mission of NIEHS to

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Galvin, M.J. and M.J. Ortner: Effects of 2450 MHzmicrowave radiation on concanavalin A or inophore induced histamine release from rat peritoneal mast cells. Int. J. Rad. Res. 39(6): 671-675, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50042-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Comparison of Impact Noise and Continuous Noise Effects on Cochlear Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although the physiological effects of continuous noise on cochlear function are well documented, the effects arising from impact noise exposure have not yet been characterized. The purpose of this project is to compare the electrophysiological changes occurring during exposure to energy equivalent impact or continuous noise exposure in guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Impact noise with peak sound pressure of 132 dB SPL and a B duration of 29 msec was generated by a mechanical impact noise generator. Continuous broad band noise of equal energy to a given intensity of impact noise was generated by a loud speaker system. Conscious guinea pigs with implanted round window electrodes, or anesthetized guinea pigs with differential electrodes placed in the perilymphatic space, were exposed to a 20 min period of impact noise or broad band noise of equal energy. The suppression of tone induced responses (cochlear microphonics and action potentials) during and 1 hour following noise exposure was monitored by presenting 6 kHz test tones.

MAJOR FINDINGS AND PROPOSED COURSE:
A. Acute preparations. When anesthetized guinea pigs were exposed to continuous broad band noise at 105 dB SPL for a period of 20 min, the cochlear microphonics (CM) was suppressed to approximately 85% of the preexposure level while the action potential (AP) was totally abolished. After noise exposure the CM recovered rapidly and the AP recovery commenced rapidly but became slower and was still incomplete after 1 hour. The changes in the tone-induced responses resulting from impact noise exposure at $L_{eq\text{-}lin}$ level of 105 dB were different from those resulting from exposure to continuous level. CM suppression was much greater, falling to 25% of the preexposure level. Suppression of AP was less than had been observed during continuous noise exposure. CM and AP recovery commenced immediately after impact noise exposure. The input-output function of CM recorded after 20 min exposure to continuous noise at 105 dB SPL showed no marked changes relative to the preexposure function, while the entire AP I/O function was suppressed. In contrast, after impact noise exposure the entire CM I/O function was reduced, while only the AP in response to low level stimuli was suppressed.

When the effects of impact noise at 122 dB_{peak} SPL presented at the rate of 1.2/s were compared with the effects of impact noise of 132 dB_{peak} SPL delivered at 0.12/s ($L_{eq\text{-}lin}$ level of 95 dB), the differences between the mean suppression of CM and AP were not significant. Even at this lower noise level exposure to continuous noise produced significantly greater suppression of the AP than did impact noise of the same energy content.

B. Chronic preparations. The pattern of response suppression resulting from exposure to continuous noise was qualitatively similar to that observed in immobilized acute preparations. The AP was found to be suppressed to a greater extent than CM. In similarity with acute preparations CM and AP appeared to be equally suppressed by impact noise exposure.

Our results indicate that the degree of cochlear response suppression produced by impact and continuous noise of equal energy is not equivalent under short-term exposure conditions. It is not possible to infer from these data whether the differences would persist during long-term exposure. A pneumatically driven impact noise generator has been developed to improve durability.

Using a pneumatically driven noise generator and electrocochleography in chronic preparations, effects of exposure to impact or continuous noise of long duration will be compared with those found in short term experiments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The equal energy hypothesis has been widely employed in the development of European noise regulations. Our results indicate that the measurement of energy may not be adequate to predict hearing loss in situations where impact noise is present. Further study of the relationship between hearing loss and the parameters of impact noise exposure are essential for the development of adequate noise regulations.

PUBLICATONS

Salt, A.N., Konishi, T., Cook, R.O. and Akay, A.: Comparison between the effects of continuous and impact noise on cochlear potentials in guinea pigs. J. Acoust. Soc. Amer. 69: 1746-1752, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50045-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Relationship of Catecholamine Levels and Fetolethality in Noise Exposed Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
	Peter Nawrot	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, P. O. Box 12233, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since a previous experiment revealed that late stage pregnancy exposure of CF-1 mice to high frequency noise resulted in a significant increase in fetolethality, the hormonal/biochemical correlates of this effect were sought. Since corticosterone levels were measured in a previous experiment (see Z01 ES 50044-02 LEB) and found to be unaffected by noise exposure, this experiment focused on catecholamines, Exogenous introduction of these substances has been found to increase fetolethality. Exposure period was 12 hours (noon to midnight) and noise was a swept band at 18-20 KHz. Reproductive-teratogenic effects determined by standard techniques included lower maternal and fetal weights increased entire litter resorption, and a significant increase in the total number of malformed fetuses. A suggestive increase in plasma epinepherine levels was noted ($p < 0.06$) and a significant increase in plasma norepinepherine levels was associated with late stage exposure. Uterine norepinepherine levels were not affected when measured on days 1 and 6 of gestation but were significantly elevated when measured on days 15.

PROJECT DESCRIPTION

METHODS EMPLOYED: Noise exposure: Mated mice assigned to the noise exposed groups were exposed from noon to midnight to a cycled high frequency tone which swept from 18 KHz to 20 KHz in 3 seconds then repeated the process after a 100 msec delay. The noise source was a device commerically marketed for repelling feral rodents. A completed description of the sound production and monitoring system and of the spectral characteristics of the sound is contained in an earlier report. There were two noise exposed groups. One group was exposed from day 1-6 of gestation and a second group was exposed from day 6-15. Both groups were housed in one chamber from day 1-18 of gestation or until sacrificed.

Teratology study: On day 18 of gestation the dams from each group (control, noise exposed days on 1-6, or days 6-15) involved in the teratogenicity study were killed by cervical dislocation and their reproductive status was noted. The conceptus at each site was examined and classified as being resorbed, dead or alive. The uteri of females designated as non-pregnant on the basis of visual examination were immersed in a 10% solution of ammonium sulfide for at least 10 minutes to identify early entire litter resorptions. The full-termed fetuses (live and dead) were sexed, weighed, and examined for external malformations. Any fetus weighing less than 0.5g or less than two-thirds the average of the larger litter mates were termed stunted. At least one-third of the fetuses of each litter were examined for visceral alterations. In addition, all stunted fetuses, and those with external malformations were examined for visceral alterations. The heads of the fetuses examined for visceral alterations were severed, fixed in Bouin's solution, and subsequently were examined by the free-hand razor section technique. All live and dead fetuses were cleared with KOH, stained with Alizarin Red and examined for skeletal alterations.

Catecholamine study: Blood and uterine samples were obtained on days 1 and 6 of gestation from the group exposed to noise on days 1-6; and on days 6, 10 and 15 of gestation from the group exposed to noise on days 6-15; and on days 1, 6, 10 and 15 of gestation from the control group. One each collection day blood and uterine samples were obtained at 3 invariant times (12:15 PM, 2:15 PM, and 4:15 PM). For the noise exposed groups, these collection times occurred 15 minutes, 135 minutes, and 255 minutes after initiation of noise exposure on day of collection. In the collection procedure, mice were anesthesized by intraperitoneal injection of 3 mg/kg pentobarbital sodium and the blood samples were taken after incision from the subclavian arteries directly into cold heparimized collecting tubes. To prevent oxidation of catecholamines, blood was immediately mixed with reduced gluthathione (approximately 2 mg of gluthathione per 1 ml of blood) and the tubes placed in an ice bath. The blood samples were centrifuged at 4°C to separate plasma from cells. The plasma was removed, placed in a screw-top vials, frozen on dry ice, then stored at -80°C until assayed for catecholamines. Immediately after blood sample collection the uterus was dissected, opened, and implantation sites removed. The uterine tissues were place in screw-top vials and frozen on dry ice for subsequent determination of catecholamine level. Blood and uterine samples were retained only from females determined to be pregnant.

Uterine catecholamine levels were measured by an HPLC method based on modifications of a technique described by Keller et al. (1976). Modification consisted

of the use of pre-extracted (with alumina) plasma as standard curve vehicle. The zero corrected standard curve using this modification was accurate at ± 20 picograms wherease when acid or water alone was used, accuracy was on the order of 200 picograms.

Plasma catecholamine levels were measured twice for each sample; once by the HPLC method described above and once by a radioenzymatic method modified.

MAJOR FINDINGS AND PROPOSED COURSE: Although the late stage of fetolethality response of the CF-1 mouse to this noise paradigm was not duplicated by the maintenance CD-1 mouse, the latter demonstrated a significant increase in the total number of malformations in addition to the early stage resorption, slightly reduced pregnancy and reduced fetal and maternal weight responses noted in most other experiments in this series. Of nine groups of pregnant mice exposed in experiments spanning five years, pregnancy maintenance was lower in all noise exposed groups relative to controls, while teratogenic responses and late stage fetolethality occurred only once, suggesting that interference with pregnancy maintainence is a highly probable result of noise stress while overt teratogenicity or late stage fetolethality require synergism with with some unknown, possibly genetic, factor.

Although this experiment is apparently the first in which catecholamine levels have been measured in the plasma and uteri of pregnant mice, the course of uterine norepinepherine (NE) levels in controls was similar to that observed in other species (rats and guinea pigs). Noise exposure corresponded to failure of the normally rapid reduction in uterine NE levels during the last half of gestation. The significance of this development cannot be assessed from this experiment, but it is interesting to note that the normal rise in plasma corticosterone between days 10-15 corresponds to the same period in which uterine NE levels are normally in rapid decline. Experiments where catecholamines have been exogenously applied have shown that these substances interfere with implantation possibly through tubal motility changes, and with the conceptus after implantation with effects ranging from reduced fetal weights at near physiological levels to fetal death at high levels. As a result, future experiments should investigate the effect of more precisely timed noise applications immediately preceding implantation, and during very late stage pregnancy (days 15-18). The latter has not been investigated during noise experiments. This project has been completed.

SIGNIFICANCE TO BIOLOGICAL RESEARCH AND PROGRAMS OF THE INSTITUTE: Identification of the hormonial/biochemical correlates of the non auditory effects of noise is a necessary step toward understanding these effects sufficiently for predictive purposes.

PUBLICATIONS

Nawrot, P., Cook, R.O. and Hamm, C.W.: Embryotoxicity of broad band high frequency noise in the CD-1 mouse. J. Tox. and Envir. Health 8: 151-157, 1981.

Cook, R.O., Nawrot, P.S. and Hamm, C.W.: Effects of high frequency noise on prenatal development and maternal plasma and uterine catecholamine levels in the CD-1 mouse. Tox. and Applied Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50046-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Chemically Induced Photosensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Colin F. Chignell	Chief	LEB NIEHS
OTHERS:	Ann Motten	NRS Postdoctoral Fellow	LEB NIEHS
	Krzystof Reszka	Visiting Fellow	LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the role played by light induced free radicals in chemically induced skin photosensitivity. Irradiation of the following drugs in aqueous or organic solution has been shown to produce a variety of free radicals: sulfanilamide, sulfacetamide, sulfadiazine, carbutamide, tolbutamide, benoxaprofen and musk ambrette. These photoinduced free radicals may play an important role in the phototoxic and photoallergic properties of these compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Many chemicals are known to cause photosensitivity in certain individuals. The photosensitive response may be one of two types, either phototoxic or photoallergic. The phototoxic reaction generally occurs during a subject's first exposure to light after the administration or topical application of a chemical and usually takes the form of an exaggerated erythemal response ("sunburn"). Photoallergic individuals may also exhibit an initial erythemal reaction. As this subsides, delayed abnormal responses begin to appear including papular, eczematous and urticarial reactions. Such reactions to light may persist for months after avoidance of the photoallergen.

While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanism of photoxicity and photoallergy is unknown. In this investigation, we have employed spin-trapping agents (2-methyl-2-nitrosopropane and 5,5-dimethyl-1-pyrroline-N-oxide) to detect the formation of free radicals during the light irradiation of several photosensitizing drugs. The structures of the trapped radicals, and the initiating free radicals have been determined with the aid of electron spin resonance. The fate of photosensitizing compounds in hairless mouse skin has also been studied using photoacoustic spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Sulfanilamide and related sulfonamides: The following radicals were trapped during the photolysis of sulfanilamide in aqueous solution: $H^{\cdot}$ and $HNC_6H_4SO_2NH_2$ (α-fission), SO_2NH_2 and $C_6H_4NH_2$ (γ-fission), $H_2NC_6H_4SO_2$ and NH_2 (δ-fission). Although the $C_6H_4SO_2NH_2$ and the SO_3^- radicals were also detected these were not formed directly by homolytic bond fission. Homolytic bond fission was also observed during the irradiation of sulfacetamide (α,δ), sulfadiazine (α), carbutamide (α,δ) and tolbutamide (δ). All of the analogs, with the exception of tolbutamide, generated the SO_3^- radical. Sulfacetamide, sulfadiazine and carbutamide generated the $C_6H_4SO_2NHR$ radical by some process that did not involve homolytic bond fission. These studies are complete and no further work is contemplated with these compounds.

Benoxaprofen: Benoxaprofen [2-(p-chlorophenyl)-α-methyl-5-benzoxazole acetic acid] is an anti-inflammatory drug that is known to cause acute photosensitivity in many patients. Irradiation of benoxaprofen in aqueous and organic solvents has provided evidence for two pathways of photodegradation. In the absence of oxygen the drug undergoes decarboxylation to form a carbon-centered radical:

$$RCHCH_3COOH \xrightarrow{e^-} R\dot{C}HCH_3 + CO_2$$

When oxygen is present a peroxy radical if formed:

$$R\dot{C}HCH_3 + O_2 \longrightarrow R\dot{C}(O\text{-}O^{\cdot})HCH_3$$

The photosensitivity induced by benoxaprofen may involve lipid peroxidation initiated by these free radicals. However, other experiments have shown that both superoxide and singlet oxygen are also produced during the irradiation of ethanolic or benzene solution containing the drug. These studies will continue in an effort to determine more precisely the mechanism of photosensitization produced by benoxaprofen.

Musk Ambrette: Musk ambrette (2,6-dinitro-3-methoxy-1-methyl-4-t-butylbenzene) is a common component of perfumes and soaps, and is also known cutaneous photosensitizer. Irradiation of musk ambrette in methanol with near UV light produces two radical species which exist in slow equilibrium with each other. Both are nitro anion radicals; one has a planar nitro anion group and in the other the nitro anion group is twisted out of the plane of the benzene ring. These assignments were made using the ESR spectra of normal and isotopically substituted musk ambrette.

Several of the known photolysis products of musk ambrette are consistent with nitro anion radical intermediates. Two other commonly used musk compounds, musk xylene (2,4,6-trinitro-1,3-dimethyl-5-t-butylbenzene) and musk ketone (2,6-dinitro-3,5-dimethyl-4-acetyl-t-butylbenzene) also produce nitro anion radical intermediates on photolysis, all of which appear to have twisted nitro anion group structures. The nitro anion radicals may be responsible for the cutaneous photosensitization reactions of musk ambrette. It is not known whether musk ketone and musk xylene also produce photosensitization.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many drugs and other environmental chemcials are known to cause skin photosensitization. These studies will help to provide a molecular basis for understanding this toxic effect. When the mechanism of photosensitization is known it may be possible to design a test which will identify those chemicals which could have potentially harmful effects on the skin in the presence of light.

PUBLICATIONS

Chignell, C.F., Kalyanaraman, B., Sik, R.H. and Mason, R.P.: Spectroscopic studies of cutaneous photosensitizing agents II Spin trapping of photolysis products from sulfanilamide and 4-aminobenzoic acid using 5,5-dimethyl-1-pyrroline-1-oxide (DMPO). Photochem. Photobiol. 34: 147-156, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50048-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Characterization of Lung Lamellar Bodies Using Spin Labels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	Gary E.R. Hook	Research Chemist	LPFT	NIEHS
	Judson Spalding	Research Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)
Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An electron spin resonance probe (5-doxyl methyl stearate) has been used to characterize the fluidity of lung Type II cell lamellar bodies which store pulmonary surfactant. These lecithin containing multilaminate structures were considerably less rigid than erythrocyte ghosts or normal leukocyte membranes, and were not changed by freezing or sonication. The lamellar bodies showed a temperature-dependent fluidity profile which was identical to liposomes made from lamellar body - extracted lipids; however it differed significantly from liposomes made of pure dipalmitoyl lecithin (DPL). Furthermore, liposomes made from a DPL-phosphatidyl glycerol combination which closely resembled the major lamellar body phospholipid components, were significantly different from the natural lamellar body liposomes. This indicates that perhaps minor phospholipids may play an important role in determining the molecular order maintained within the lamellar bodies.

PROJECT DESCRIPTION

METHODS EMPLOYED: The properties of lamellar bodies from rabbit lung type II cells have been investigated using electron spin resonance (ESR) probes. The lamellar bodies were extracted and purified according to well established procedures. The spin label (5-doxyl methyl stearate) was purchased from Syva Associates, Palo Alto, California. ESR measurements were taken using a Varian E-109 X-band spectrometer equipped with a E-238 Tm_{110} cavity and a variable temperature apparatus.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Artificial Systems. The effect of temperature on the motion of 5-doxyl methyl stearate (5 DMS) in liposomes made from pure dipalmitoyl lecithin (DPL) was investigated. At high temperature (52°C - 41°C) the probe reported an almost constant fluidity; however between 41°C and 38°C a sharp transition point occurred. The increase in fluidity of the probe then progressed in a linear manner as far as 22°C. Liposomes made from egg phosphatidyl choline also showed a similar transition temperature; however the increase in fluidity as a function of temperature was much less than the DPL liposomes. We have also examined phosphatidyl glycerol ((PG) which constitutes about 12% of the lamellar body phospholipids) and DPL/PG combination liposomes. The PG liposomes bound the spin label in a more highly immobilized manner than the DPL liposomes at temperatures between 45°C-21°C and the combination liposomes were intermediate. 2. Lamellar Bodies. The effect of temperature on the motion of 5-DMS in extracted lamellar bodies was studied. The probe showed a fluidity in the lamellar bodies which was closer to the artificial systems and much higher than human erythrocyte ghosts or normal leukocyte membranes. The effect of temperature showed a slight transition point at 40°C and an additional transition at 28°C. Lamellar bodies which had been sonicated or frozen were similar to the native state organelle. In further experiments, mixed lipids were extracted from the lamellar bodies using chloroform/methanol (2:1), and liposomes were formed. The motion of the spin label in the liposomes was unchanged from that of the native state lamellar bodies at temperatures between 50°C-22°C.

Although the motion of the spin label in the lamellar bodies was closer to that of the artificial systems than that of ghost or leukocyte membranes, it was significantly different from the artificial liposomes composed of the major constituent phospholipids (DPL/PG). This finding indicates that perhaps a minor lipid component may be influencing the structure of the lamellar bodies.

These spin probe studies are being currently expanded to study other artificial systems including some of the minor lipid components of the lamellar bodies. We hope eventually to duplicate the natural fluidity profile and thereby determine the effect of minor components of the lamellar bodies. In addition, we are currently isolating sufficient quantities of lamellar bodies to isolate and separate the component lipids to determine the effect of each on the temperature profile of the interacalated spin label. When these studies are completed the effect of exposure to oxidizing gases (SOx, NOx) on the molecular structure of the lung lamellar bodies will be examined. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The acellular layer of surfactant which is essential for normal pulmonary function is stored by the lamellar bodies of Type II cells. These lecithin-rich organelles also contain hydrolytic enzymes and may be a source of the hydrolases secreted into the acellular lining of the branchioles. Although the lipid components of these airways have been isolated and characterized, there has been no thorough study of the interaction of these components with one another within the lamellar body. This study should provide information leading to a more complete characterization of the molecular structure of this organelle which is essential to normal pulmonary function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50050-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

ESR Evidence for a Free Radical in the Cis-Trans Isomerization of Furylfuramide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been proposed that the enzymatic cis-trans isomerization of furylfuramide is the result of anion free radical formation by nitroreductases. Electron spin resonance measurements of the furylfuramide anion free radical have provided direct spectral evidence for this intermediate, and clarified the disputed relationship between the isomerization and the nitro reduction of furylfuramide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectra of anaerobic microsomal incubations at 25° were obtained with a Varian century series E-109 spectrometer equipped with a TM_{110} cavity. Visible spectra of *cis*- and *trans*-furylfuramide (AF-2) were obtained with a DW-2A Aminco-Chance spectrophotometer at 37°.

MAJOR FINDINGS AND PROPOSED COURSE: Tatsumi *et al.* have proposed that enzymatic *cis-trans* isomerization of furylfuramide is a direct consequence of enzymatic nitro reduction. The nitroreductases, which are inhibited by oxygen, transfer a single electron to nitro substrates to give their respective anion free radicals. The carbon-carbon double bond linking the two furan rings of AF-2 would be weakened by anion radical formation, because the additional electron is in an antibonding molecular orbital. Upon formation, the *cis*-AF-2 anion free radical was proposed to isomerize rapidly to the *trans*-AF-2 anion, which could then be oxidized to form *trans*-AF-2.

Recently, Tomoeda and Kitamura studied the isomerization and nitro reduction of *cis*-AF-2 by subcellular fractions of *E. coli*. Their observations suggested that the nitro reducing and isomerizing activities of *E. coli* were due to totally different enzymes, in apparent contradiction of the isomerization mechanism of Tatsumi *et al.*

The ESR spectrum of an anaerobic microsomal incubation containing furylfuramide and an NADPH-generating system provides direct evidence of free radical formation. In the presence of air, nitroaromatic anion free radicals undergo rapid air oxidation to form superoxide anion, and the nitroaromatic anion free radicals not detected. In addition, identical spectra were obtained with either *cis*- or *trans*-AF-2. These ESR spectra did not vary with time even though visible spectroscopy shows a rapid and nearly complete conversion of *cis*-AF-2 to *trans*-AF-2.

Although spectral evidence alone indicates that both the major and the minor components of the spectrum represent AF-2 anion free radicals, the assignment of the spectrum to *cis-trans* anion free radicals must be made on other grounds. INDO (intermediate neglect of differential overlap) molecular orbital calculations on *cis*- and *trans*-AF-2 anions suggest that the *cis* anion radical will have the larger nitrogen hyperfine splitting constant. Furthermore, the greater steric hindrance expected for the *cis*-conformer should result in a predominance of the *trans*-conformer. In the parent compounds, the greater thermodynamic stability of *trans*-AF-2 (87-91%) vs that of *trans*-AF-2 to *cis*-AF-2 (7-11%) observed in xanthine oxidase incubations under optimum conditions. Clearly, since xanthine oxidase is only a catalyst, and cannot change the thermodynamic equilibrium of *cis*- *trans*-AF-2, the equilibrium ratio of *cis*- to *trans*-AF-2 and the two free radicals is the strongest chemical evidence for our assignment of the two species as the respective anion radicals. The observation of distinct conformational isomers with ESR only requires that the equilibrium mixture be slowly interconverting on the ESR time scale, which is very rapid. More precisely, the lifetime of the conformers must be much longer than $1/\gamma_e (a^N_{cis} - a^N_{trans}) = 28$ nanoseconds.

The reaction can still be fast in terms of chemical reaction, and in fact, competes favorably with the autoxidation of the AF-2 anion free radicals.

The results of Tomoeda and Kitamura and the isomerization mechanism of Tatsumi *et al.* are not contradictory if the oxygen-insensitive *E. coli* reducing activity does not form the anion radical intermediate and the oxygen-sensitive *E. coli* reducing activity does form the anion radical, as has been recently demonstrated. In the present study, we provide electron spin resonance evidence for *cis*- and *trans*-AF-2 anion radical intermediate formation during the microsomal *cis-trans* isomerization of AF-2.

Higher resolution ESR investigations in D_2O buffer, in conjunction with INDO calculations, support this concept by unambiguously demonstrating the enzymatic generation of *cis* and *trans* radical anions of 3-(5-nitro-2-furyl)-2(2-furyl) acrylamide. The INDO calculations further indicate that the rotational barrier between the *cis* and trans anion radicals of this compound is only 5-10 kcal/mole, whereas a 70 kcal/mole barrier exists for the parent geometric isomers. Hyperfine splitting constants for the *cis-trans* conformers have been assigned on the basis of INDO calculations. Surprisingly, only the nitrogen hyperfine splitting of the nitro group is distinguishably different in the two conformers, a result which is not inconsistent with the INDO calculations. This project has been completed and no further work is planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Furylfuramide has been shown to be mutagenic, carcinogenic and cytotoxic. Enzymatic nitro reduction of furylfuramide is thought to form the reactive metabolites which are primarily responsible for these effects. The synthetic form of this former food additive is the *cis* isomer. Typical mammalian nitroreductases, such as xanthine oxidase or rat liver microsomal nitroreductase, isomerize *cis*-furylfuramide to *trans*-furylfuramide before they initiate reductive "activation". Many investigators have observed this isomerization in the course of investigations of the reductive activation of *cis*-AF-2 by mammalian and bacterial systems.

PUBLICATIONS

Kalyanaraman, B., Mason, R.P., Rowlett, R., and Kispert, L.D.: An electron spin resonance investigation and molecular orbital calculation of the anion radical intermediate in the enzymatic cis-trans isomerization of furylfuramide, a nitrofuran derivative of ethylene. Biochim. Biophys. Acta 660: 102-109, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50051-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Free Radical Metabolism of Polycyano Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polycyano compounds have been proposed for use as superconductors in high voltage transmission lines. These compounds are known to be strong electron acceptors which also form charge-transfer complexes. It is the objective of this study to examine the biological properties of polycyano compounds and to determine their metabolic fate. Preliminary experiments have shown that in microsomal incubations the electron transfer between tetracyanobenzene and some unknown biological donor is complete and the radical anion of this compound is formed. The electron transfer to form the tetracyanobenzene anion radical is dependent upon the presence of NADPH. In future studies, the source of the electron will be sought and the microsomal metabolism of other polycyano compounds will be examined.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy was employed to detect steady-state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: Tetracyanobenzene was metabolized to an anion free radical by rat liver microsomes. The ESR spectrum of this free radical metabolite was dependent upon NADPH. We now intend to determine what other metabolites are formed by this reduction path, because microsomal incubations acquire a pink color due to a metabolite, which is not a free radical. Cyanide is also expected to be a reduction metabolite, because it could form by reductive cleavage of the phenyl-nitrile bond. This project is not progressing due to our inability to obtain pure tetracyano benzene.

Next, we will investigate the source of the single electron which was transferred to tetracyanobenzene. The main possibilities are cytochrome P450 and b_5 and their respective flavin-containing reductases, NADPH-cytochrome P-450^5 and NADPH-cytochrome b_5 reductase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tetracyanocompounds are used in organic superconductors, which are thought to have great potential in the transmission of high voltages without power loss. Although these compounds are reported to have fungicidal and bactericidal properties there is little or no information on their other biological effects and nothing is known about their toxicity and metabolic fate.

PUBLICATIONS

Mason, R.P.: Free radical metabolites of toxic chemicals. In Pryor, W.A. (Ed.): Free Radicals in Biology, Vol. V, New York, Academic Press, 1982, pp. 161-22.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50052-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Binding of Chemically Activated Semiquinone Free Radicals from Anticancer Agents to DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chemical reduction of the highly active quinone-containing antitumor drugs, adriamycin and daunorubicin formed the same partially reduced free radical previously reported by microsomal activation. *In vitro* incubation of the chemically activated free radical intermediates with DNA resulted in covalent binding of these drugs to DNA. The adriamycin semiquinone radical has a greater affinity for DNA and covalent complexes containing up to one adriamycin per 15 nucleotides were obtained. The daunorubicin semiquinone radical, on the other hand, showed a lesser binding affinity and gave rise to complexes in which one drug molecule was covalently bound per 140 nucleotides. Studies with synthetic polynucleotides suggest that these drugs have a high preference for poly (dG) and poly (dC). Microsomal activated drugs also bind covalently to DNA with identical binding affinities. Adriamycin, when injected in rats, also bind covalently to rat liver proteins, RNA and DNA. Microsomal activation of these drugs produced both C_7-free radical and C_7-quinone methide which act as alkylating agents.

PROJECT DESCRIPTION

METHODS EMPLOYED: We have previously shown that chemical reduction ($NaBH_4$) of adriamycin and daunorubicin generated same free radical intermediates as previously reported by microsomal activation. Incubation of the free radical intermediates with DNA, in vitro, resulted in covalent binding of these drugs to DNA. Adriamycin had a greater binding affinity for DNA than daunorubicin which correlates with their ability to indule sister chromatid exchanges. We have also shown that the microsomal-NADPH activated drugs covalently bind to nucleic acids and that this binding decreases with increasing incubation time prior to the addition of DNA.

MAJOR FINDINGS AND PROPOSED COURSE: Recently we have examined the binding of ^{14}C-adriamycin to cellular macromolecules in vivo. Intraperitoneal injection of ^{14}C-adriamycin to rats results in covalent binding of the durgs to protein, RNA and DNA. Our results show that more adriamycin is bound to protein and to RNA than DNA at each point. Furthermore, the binding decreases rapidly with time indicating that an enzymatic repair process may be operating. Although, the species that bind(s) to the cellular macromolecules is not known, Moore and we have suggested that C_7-quinone methide and/or C_7-free radical metabolite of adriamycin may act as active alkylating species. Recently, we have examined the formation and binding of these one electron (C_7-free radical) and two electron (C_7-quinone methide) reduction products of adriamycin and daunorubicin. Our findings show that microsomal-NADPH reduction of these drugs produces both one and two electron reduction product. Furthermore, the C_7-quinone methide also binds to DNA. Future plans include characterization of the adduct by enzymatic degradation and a study of the binding of C_7-free radical and C_7-quinone methide to soluble SH-compounds (GSH, cystein).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the mechanism of action of antitumor drugs and chemical carcinogens stems from their ability to interact with nucleic acids, it is of great significance to understand such binding at a molecular level. It is hoped that by defining these interactions, a better understanding of the tumorigenicity will result.

PUBLICATIONS

Sinha, B.K.: Binding specificity and chemically and enzymatically activated anthracycline anticancer agents to nucleic acids. Chem. Biol. Interact. 30: 67-77, 1980.

Sinha, B.K. and Gregory, J.L.: Role of one-electron and two-electron reduction products of adriamycin and daunomycin in deoxyribonucleic acid binding. Biochem. Pharmacol. 30: 2626-2629, 1981.

Sinha, B.K.: Myocardial toxicity of anthracyclines and other antitumor agents. In Vanstee, E.W. (Ed.): Cardiovascular Toxicology. Raven Press, 1982, pp. 181-197.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50053-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Binding of Antitumor Drugs to Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cytotoxic and mutagenic properties of antitumor drugs such as adriamycin, acridines, diacridine, actinomycin D and Pt compounds are related to their interaction with nucleic acids and inhibition of protein systhesis. We have examined their interaction with human erythrocyte ghost membranes and murine mastocytoma cells using spin labeling techniques. These drugs induce changes in electron spin resonance of the spin labeled ghost membranes and in the mastocytoma cells. These findings suggest that these drugs induce changes in protein conformation of the membranes. Using cytofluorescence technique, we have shown that the binding of adriamycin leads to the protein aggregation in Hut-II cells. Thus, membrane binding properties of these drug may be important in their mechanism of action.

PROJECT DESCRIPTION

METHOD EMPLOYED: In previous studies we have shown that a number of antitumor drugs (which bind to nucleic acids and are also carcinogenic), interact with membranes. We also found, using spin labeling techniques, that these drugs induce changes in red cell ghost membranes and mastocytoma P815 cells suggesting alteration of their protein conformation. These findings may be related to the cytotoxicity of these agents since alterations in protein conformations may be expected to affect many physiological functions (ions transport, glucose transport, etc) of the cells.

MAJOR FINDINGS AND PROPOSED COURSE: We have now examined the binding of anthracycline antibiotics, adriamycin and daunomycin, to cell membranes using fluorescence microscopy. Incubation of the drugs (10^{-6}M, 10^{-5}M) with human ghost membranes, followed by removal of the unbound drugs by washing, resulted in a bright yellow membrane fluorescence indicating that the drugs were bound. In live cells (mastocytoma 815 and Hut II), incubation of either adriamycin or daunomycin for 5 min at 37°C resulted in a faint yellow fluorescence apparently associated with the nuclear membranes. Furthermore, only a bright yellow fluorescence was detected after 4 hrs of incubation. These observations do not support earlier observations of Krishan, et al. and Bachur, et al. who have shown that these drugs bind to nucleic acids and appear as red-orange fluorescence. Furthermore, Krishan, et al. had shown that this adriamycin-specific red-orange fluorescence appears readily in dead cells. Our observations then suggest that either the binding of adriamycin does not lead to cell death or the binding does not produced red-orange fluorescence. Counterstaining with trypan blue, which is excluded by the live cells, however, resulted in both yellow and red fluoresence. Furthermore, the yellow fluorescence was exclusively associated with the live cells while the red fluorescence was observed in the dead cells. In addition, a distinct patching of the yellow fluorescence was detectable in these cells suggesting that the binding of adriamycin leads to protein aggregation. The observation that the red fluorescence is always associated with cells that have taken up trypan blue suggests that this red fluorescence originates from trypan blue which is excited via non-radiative energy transfer from adriamycin. This project is essentially complete and no further work is planned at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the carcinogenic and mutagenic properties of antitumor drugs are most likely associated with DNA binding, it is of great significance to understand the mechanism of actions of these drugs at a molecular level. It is hoped that such studies may lead to better understanding of the chemical properties that are responsible for the carcinogenic and antitumor properties of these agents.

PUBLICATIONS

Sinha, B.K. and Cysyk, R.L.: Mechanism of action of N^2-substituted spin labeled actinomycin D binding to nucleic acids and erythrocyte ghost membranes. Chem. Biol. Interact. 34: 367-372, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50054-04 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Free Radical Formed Microsomal Incubations Containing CCl_4 and NADPH

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.P. Mason	Research Chemist	LEB	NIEHS
OTHERS:	C.F. Chignell	Chief	LEB	NIEHS
	C.R. Wolf	Visiting Associate	LP	NIEHS
	R.M. Philpot	Research Chemist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hepatotoxicity of carbon tetrachloride is usually thought to be due to the enzymatic formation of the trichloromethyl radical. A variety of indirect, but not conclusive, evidence for the formation of $^{\cdot}CCl_3$ exists: hydrogen abstraction by $^{\cdot}CCl_3$ to form $CHCl_3$ and dimerization of $^{\cdot}CCl_3$ to form C_2Cl_6. Hydrogen abstraction of a methylene hydrogen from polyunsaturated fatty acids by the trichloromethyl radical would be followed by oxygen addition and would result in lipid peroxidation. Carbon tetrachloride-induced lipid peroxidation has been extensively studied both in vitro and in vivo. Attempts to use electron spin resonance (ESR) spectroscopy to demonstrate directly the presence of the trichloromethyl radical in hepatic microsomal incubations or liver slices have been unsuccessful. Recently a free radical has been detected in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-t-butyl nitrone (PBN). This free radical adduct was identified as the $^{\cdot}CCl_3$ adduct of PBN. Our studies have shown, however, that a lipid dienyl radical, similar to that formed by the action of soybean lipoxygenase on linoleic acid, is the species being trapped.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy, in conjunction with spin-trapping agents, has been used to detect steady state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: With the aid of the spin-trapping technique, Poyer, et al. have detected a free radical in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-t-butyl nitrone (PBN). This free radical was identified as the trichloromethyl adduct of PBN based on the similarity of its ESR spectrum to that of the free radical formed by UV photolysis of a CCl_4 solution of PBN. Ingall et al. using the spin trap 2-methyl-2-nitroso-propane (MNP), have also trapped a free radical in microsomal incubations containing CCl_4 and NADPH, but in this case, the spectrum was not the same as that generated by the X-ray irradiation of a CCl_4 solution of MNP. The latter spectrum was claimed to be that of the MNP-trichloromethyl spin adduct based on an isotopic effect seen with ^{13}C-carbon tetrachloride. However, it should be noted that this spectrum of the MNP-trichloromethyl radical adduct is clearly different from earlier spectra in that hyperfine structure due to chlorine was not observed. Based on the differences between the microsomal and X-ray irradiation spectra, Ingall et al. concluded that the free radical trapped in the microsomal experiments was probably either CCl_3-$O_2^{\cdot}$ or a secondary lipid peroxyl radical rather than the $^{\cdot}CCl_3$ radical. Our spin-trapping investigations with PBN and MNP have demonstrated that these interpretations are in error, and that in both cases a lipid dienyl free radical, similar to that formed by the action of soybean lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) on linoleic acid, is probably the species that is trapped.

Further investigations with MNP and PBN are in progress. Preliminary results indicate the MNP-carbon centered lipid adduct may be a result of a non-enzymatic nonfree radical reaction of MNP with the microsomal lipids.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: CCl_4 toxicity results from metabolic activation of this agent by the liver, which is the main site of CCl_4-induced pathological changes. This activation is thought to require the homolytic cleavage of one of the chlorine-carbon bonds of CCl_4, to form the trichloromethyl free radical. From this single unproven event the entire spectrum of pathological consequences of CCl_4 poisoning is thought to follow. The central importance of this proposed free radical metabolite to the hepatotoxicity of CCl_4 makes a demonstration of its existence in a biological system of considerable importance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50056-03 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of hypothermia on ion movement in guinea pig cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	LEB	Medical Officer (Research)
	Alec N. Salt	LEB	Visiting Fellow
OTHER:	Philip E. Hamrick	SFTY	Radiation Safety Officer

COOPERATING UNITS (if any)

Radiation Safety

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been documented that the cochlear potentials are maintained by metabolic energy. However, it is not known whether a decrease of metabolic rate by hypothermia can alter ion movement or membrane permeability of the cochlea. The present study is designed to determine dependence of cochlear membrane permeability on temperature.

PHS-6040
(Rev. 2-81)

diffusion. The K^+ conductance was calculated from the rate of change of the endolymph K^+ concentration relative to the K^+ electrochemical potential difference. The mean G_K averaged from 10 to 30 min after onset of anoxia was (21.53 $\pm$ 5.54)x 10^{-6}mho in hypothermic guinea pigs, whereas the mean G_K averaged during the same period was (34.85 $\pm$ 5.60)x10^{-6}mho in normal guinea pigs.

A manuscript has been accepted for publication and this project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The present data suggest that hypothermia results in changes in both active and passive ion transport mechanisms in the cochlea. It is likely that a decrease of metabolic energy caused by ototoxic insults may interfere not only with active ion transport, but also the membrane permeability of the cochlear partition.

PUBLICATIONS

Konishi, T., Salt, A.N. and Hamrick, P.E.: Effects of hypothermia on ion movement in guinea pig cochlea. Hearing Res. 4: 265-278, 1981.

PROJECT DESCRIPTION

METHODS EMPLOYED: The cochlear potentials were recorded from the basal turn of the cochlea of guinea pigs anesthetized with sodium pentobarbital. The rectal temperature and blood pressure in the common carotid artery were monitored. The rectal temperature was reduced from 39° ± 0.5°C to 29° ± 0.5°C with a cooling pad. The perfusion of the perilymphatic space with solutions containing ^{43}K and ^{22}Na and collection of the cochlear fluids were carried out during steady state hypothermia. Total concentrations of K^+ and Na^+ in the cochlear fluids were determined by a helium glow photometer and radioactivities of ^{43}K and ^{22}Na were determined by gamma spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potenitals. The endocochlear potential (EP) measured at 39°C was 86.0 ± 3.5 mV. When the rectal temperature decreased to 29°C the EP was 78.2 ± 3.5 mV. The EP recorded 2 hours after the rectal temperature was kept at 29.0° ± 0.5°C was 73.2 ± 3.9 mV. During the course of hypothermia the magnitude of cochlear microphonics (CM) gradually decreased. Usually a large increase of the negative summating potential accompanied the decrease in CM during the period of hypothermia. The action potential (AP) in response to test stimuli of low intensity were markedly suppressed but the AP remained little changed or became supernormal with high intensity stimuli.

2. Electrolyte concentrations in the cochlear fluids. Hypothermia did not result in marked changes in K^+ concentrations in the endloymph and perilymph of nonperfused cochlea. The Na^+ concentrations in both endolymph and perilymph were slightly decreased in hypothermic guinea pigs.

3. Membrane permeability of the endolymph-perilymph barrier determined by rate constant for K. When the perilymphatic space was perfused with radioactive artificial perilymph, the ^{43}K concentrations in the endolymph (normalized by ^{43}K concentrations in the perilymph) increased exponentially. The rate constant for K^+ was 0.0069 min^{-1} in hypothermic guinea pigs which was significantly lower than the value obtained in normal guinea pigs (0.013 min^{-1}).

The K^+ conductance of the endolymph-perilymph barrier can be computed by using the following equation

$$G_K = \frac{\lambda_K F^2 V_{end} [K^+_{end}]}{\Delta\tilde{\mu}_K}$$

where V_{end} is volume of the endolymph (2μl) and $\Delta\tilde{\mu}_K$ is the electrochemical potential difference between endolymph and perilymph. The computed G_K was 20.65 x 10^{-6} mho in hypethermic guinea pigs which was considerably lower than 35.16 x 10^{-6} mho in normal guinea pigs. The permeability constant for K^+ (P_K) was 133.54 x 10^{-9} cm^3 sec^{-1} in hypothermic guinea pigs. This value was substantially lower than the normal value (242.9 x 10^{-9} cm^3 sec^{-1}).

4. Membrane permeability of the endolymph-perilymph barrier determined from K^+ passive diffusion during anoxic period. Permanent anoxia was induced 30 min after the rectal temperature fell to 29.0°C. When anoxia continued for longer than 5 min the decrease of the endolymph K^+ concentration measured with K^+ selective liquid membrane electrodes was solely attributable to a passive K^+

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50057-03 LEB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Microwave Radiation on Reproductive Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Cindy H. Hall	Graduate Student	N.C. State University	
	J. Paul Thaxton	Poultry Science Dept.	N.C. State University	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle, N.C.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, the effect of non-ionizing radiation on the integrity of mature spermatocytes was examined. Semen was obtained from 10-month-old turkeys and diluted to a concentration of 3.5 x 10^8 sperm/ml using Beltsville Poultry Semen Extender. The sperm were exposed for 30 minutes to 2.45 GHz microwave radiation at specific absorption rates (SAR's) of 0, (sham) 10 or 50 mW/g. The waveguide exposure system maintained the control (non-irradiated) and irradiated samples at 40 ± 0.15°C throughout the experiment. Microwave radiation did not alter membrane permeability to a vital stain or the intracellular enzymes LDH and GOT. The in vivo performance of the sperm following in vitro irradiation was also examined. The fertility of virgin turkey hens given a single insemination of microwave exposed sperm was similar for the 10 mW/g and nonexposed sperm. Hatchability and embryonic mortality were not different among the treatment groups. A number of hematological parameters in the progeny of the turkey hens are now being examined.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: a. Cell Preparation: Semen was obtained from 7-month-old Nicholas large white turkeys. Biological variation was minimized by pooling semen from 30 turkeys. For each experiment, 2 ml of semen were collected and diluted 2:1 with Beltsville Poultry Semen Extender, BPSE (USDA, Beltsville, MD). The semen was washed twice to remove the seminal plasma. The sperm were then resuspended in BPSE to a concentration of 5.0×10^8 sperm/ml. The sperm were irradiated in an S-band waveguide chamber at a frequency of 2450 MHz. Each tube contained 4.2 ml of the sperm suspension. The tubes were siliconized to minimize cellular adhesion and were gently stirred throughout the exposure duration to allow uniform microwave absorption and temperature distribution.

b. Cellular Integrity: Several parameters were selected as indices of altered membrane function: permeability to a vital stain (viability) release of lactate dehydrogenase (LDH) and release of glutamic oxalic transaminase (GOT).

c. In vivo performance of sperm following in vitro irradiation: Virgin turkey hens were given a single insemination of sperm which were either sham-exposed, or exposed to 10 mW/g or 50 mW/g. Egg fertility was determined during the nine weeks following insemination.

d. Evaluation of progeny from turkey hens receiving microwave irradiated sperm. Hematological evaluation of the progeny from eggs laid during the second and fourth weeks after insemination. The turkey polts were evaluated a 2, 4 and 5 weeks of age.

MAJOR FINDINGS AND PROPOSED COURSE: a. Cellular Integrity: Initially, the viability was 96% and was not affected by microwave radiation at any of the exposure levels examined. The release of the soluble enzymes, lactic acid dehydrogenase, and glutamic oxalic transaminase, by sperm into the suspending media was not altered.

b. In vivo performance of sperm following in vitro irradiation: Fertility declined comparably in all groups until the fourth week after insemination. During the fourth week, fertility increased in the hens which received 50 mW exposed sperm and elevated fertility was maintained in this group for the duration of the experiment. Hatchability and embryonic mortality were not different.

c. Evaluation of progeny from turkey hens receiving microwave irradiated sperm: No data available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment are of interest to the National Institute of Environmental Health Sciences. Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control the temperature of the specimen carefully and be able to reproduce the exposure conditions. We have developed the capability to do this for in vitro exposures at NIEHS and this may provide a system for differentiating specific microwave effects from thermal responses. In addition, by examining the response of cells and cellular components to microwave radiation,

it should be easier to identify the mechanisms of action of microwave radiation with biological specimens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50058-03 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Microwave Effects on Fetal Development in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Peter Nawrot	Visiting Associate	LEB	NIEHS
OTHER:	Minuro Inouye	Visiting Fellow	LEB	NIEHS
	R.E. Staples	Research Scientist	Dupont Corp., Wilmington, Del.	

COOPERATING UNITS (if any)

Research Triangle Institute

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pregnant mice (CD-1 strain) were exposed to 2.45 GHz microwave radiation at a power density level of 30 mW/cm^2. At exposure to 30 mW/cm^2 (SAR ≃ 32 mW/g) during days 1-6 a significant decrease in implantation sites per litter and average fetal weight was observed. Exposure to 30 mW/cm^2 during days 6-15 resulted in a slight increase in the number of malformed fetuses but was not statistically significant as obtained in a previous experiment. This repeat of previous work again indicates that the threshold for teratogenic effects in the CD-1 mouse strain is approximately 30 mW/cm^2.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this research was to determine the maternal and embryotoxic effects of microwaves. In order to determine whether or not the effects were only thermal or a combination of thermal and specific microwave interactions, groups of animals were placed in elevated temperature environments in order to simulate the thermal stress of the microwave exposure. The mice were exposed from above in styrofoam cages (one animal per cage) separated at least 2 body lengths with the long axis of the cages parallel to the electric field.

MAJOR FINDINGS AND PROPOSED COURSE: We repeated the experiment which exposed pregnant CD-1 mice to 30 mW/cm^2 to 2.45 - GHz microwave radiation. Our investigation showed that exposure to 30 mW/cm^2 during days 1-6 produced a signficant decrease in implantation sites per litter and average fetal weight. In our previcus study exposure to 30 mW/cm^2 during days 6-15 resulted in a small but statistically significant increase in the number of malformed fetuses, primarily cleft palate. In this study a slight but not significant increase in malformed fetuses were observed. This supports our original contention that 30 mW/cm^2 (SAR $\simeq$ 32 mW/g) is near the threshold level for producing teratogenic effects in CD-1 mice.

The research project will be completed during this fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: Sufficient information is not available at the present time to establish scientifically based safety standards for microwave radiation exposure. The determination of the intensity of microwave radiation which produces teratogenic effects is important to the evaluation of the hazardous effects of microwaves. This research is part of the mission of the Institute to conduct research on the health effects of physical factors in the environment.

PUBLICATIONS

Nawrot, P.S., McRee, D.I. and Staples, R.E.: Effects of 2.45 GHz microwave radiation on embryofetal development in mice. Teratology 24: 303-314, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50059-03 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Protoporphyrin Phototoxicity in Rat Mast Cells and Human Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHER:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Protoporphyrin-mediated phototoxicity has been studied in human erythrocytes and rat mast cells. Circular dichroism studies of erythrocyte ghosts have shown that phototoxic lysis is accompanied by membrane protein denaturation with loss of alpha helical structure. Rat mast cells showed a dual phototoxic response which depended on the light intensity. Low intensity light caused a stabilization of the cell membrane resulting in a loss of histamine secretory ability; whereas high intensity light caused phototoxic lysis. Sodium dodecyl sulfate disc gel electrophoresis indicated that the mast cell membrane proteins are covalently crosslinked by the phototoxic reaction in a manner similar to that seen in erythrocyte ghosts. Biophysical studies are currently underway to establish the molecular mechanisms of these phenomonea.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Severe phototoxic reactions may be clinically manifested in patients with erythropoietic porphoryia, a metabolic disease which results in a high buildup of porphyrins in the blood. Porphyrin-mediated phototoxicity is related to erythrocyte hemolysis, and in spite of extensive biochemical studies, the molecular mechanism is not completely understood. We have studied this phototoxic reaction in erythrocyte ghosts using circular dichroism (CD), a technique which is sensitive to membrane protein conformation. In addition, we have studied the reaction in a eukaryotic cell which can be stimulated in vitro to perform a biological response. The purpose of this approach is to study more closely the intermediate oxidative reactions which precede erythrocyte lysis, and to determine the effects of these reactions on erythrocytes and functioning eukaryotic cells.

METHODS EMPLOYED: Purified rat peritoneal mast cells and human erythrocyte ghosts were obtained using well established methods. Cells and ghosts were exposed to light in the presence of protoporphyrin using either a 100W incandescent light bulb or a 100W mercury vapor lamp. The light intensity was varied by changing the distance from the light source. Circular dichroism measurements were performed on erythrocyte ghosts exposed to light and protoporphyrin using a Jasco automatic recording polarimeter. Simultaneous measurements of membrane protein optical density at 280nm indicated that changes in the circular dichroism spectra were not due to scattering artifacts.

MAJOR FINDINGS: Human erythrocytes and ghosts. Human erythrocytes exposed to strong light in the presence of protoporphyrin underwent lysis on a time-dependent basis. An equivalent number of ghosts exposed under identical conditions showed changes in CD spectra of the membrane proteins which were consistent with loss in α-helical structure due to protein denaturation. Appropriate controls showed that the effect was dependent both on protoporphyrin and light. Changes in the CD spectra began after 2-4 min when the ghosts (20 µg Protein/ml) were exposed to 2mM protoporphyrin and placed 20 cm from the mercury vapor light source. In the intact erythrocytes, phototoxic lysis began between 6-8 min after the begining of illumination and progressed with time in a sigmoidal fashion until almost 100% lysis occurred. The data therefore indicate that changes in the secondary protein structure of erythrocyte ghost proteins can be correlated with protoporphyrin induced photolysis.

Although D_2O decreased the rate of lysis in erythrocyte ghosts, there were no effects on the rate of decrease in membrane protein secondary structure as determined by circular dichroism. This suggests that either singlet oxygen does not play a significant role in protoporphyrin phototoxicity or that in this system, singlet oxygen does not decay via collisions with the aqueous environemnt.

Rat Peritoneal Mast Cells. Rat mast cells undergo lysis when exposed to protoporphyrin and high intensity light. This reaction is manifested by the cytotoxic release of histamine due to conditions which may be similar to those seen in erythrocyte ghosts. This study has shown, however, that mast cell membranes do not lyse when exposed to protoporphyrin and low intensity light, but rather are stabilized in a way which makes them resistant to histamine liberators. The

development of this inhibition is both dose and time dependent (100 μg/ml protoporphyrin for 30 min gives total inhibition) and does not occur in the dark or under ordinary room light. Purified mast cells were exposed to conditions which produced inhibition, and the proteins separated using SDS-disc gel electrophoresis. Preliminary results indicated that some of the proteins were unable to enter the gel, presumably due to crosslinking and aggregation. This would indicate that the stabilization phase of protoporphyrin phototoxicity in mast cells may involve a protein crosslinking similar to that which accompanies erythrocyte lysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Severe phototoxic urticaria can occur as a result of clinical disorders such as erythropoietic porphyria, from direct phototoxic reactions to drugs and chemicals or from photoallergic reactions to haptens. A biophysical study of phototoxicity in erythrocyte membranes has not been previously undertaken. Protoporphyrin-induced phototoxicity is therefore being studied as a model system in order to understand more clearly the role of active oxygen in the phototoxic response. The use of these techniques will contribute to our knowledge of both membrane physiology and phototoxicity. The mast cell provides an excellent model for both *in vitro* and *in vivo* studies of phototoxicity in a eukaryotic cell. Furthermore, mast cells secrete several mediators of inflammation and since they occur abundantly in the skin, they may play a direct role in the phototoxic response. A thorough study is therefore needed to understand the molecular mechanism of this response in the mast cell. These studies may lead to a method of control over the symptoms of phototoxic urticaria.

PROPOSED COURSE: We are currently developing techniques to study the effects of several agents on the early development of membrane protein denaturation and the involvement of specific ghost proteins. Since protoporphyrin phototoxicity is probably mediated via reduced forms of active oxygen, we have studied the early stages of phototoxicity in the presence of agents which are known to affect these forms (D_2O superoxide dismutase, mannitol, catalase, etc.) The use of circular dichrosim makes it possible to study the time course of membrane protein denaturation directly and also to study this reaction in the early, pre-lytic stages of phototoxic damage.

The effects of protoporphyrin phototoxicity on membrane intercalated spin labels will be studied using ESR. Lipid spin labels can be intercalated into erythrocyte ghosts to probe the interface regions and the hydrophobic interior. In addition, the membrane proteins may be covalently spin labeled and the phototoxic reaction studied at this level. Because the spin probes are degraded by the strong oxidants produced during the phototoxic response, it may be possible to determine the area in the membrane where the greatest number of oxidative reactions are occurring, and whether this area changes during the course of the reaction.

Protoporphyrin mediated phototoxicity has not as yet been studied directly in erythrocyte ghosts. Using the biophysical techniques outlined above, we hope to learn more about this phototoxic response and the secondary reactions, it precipitates in the erythrocyte membrane.

We plan to continue these studies of phototoxicity in mast cells by studying the

reaction in the presence of inhibitors and quenchers of reduced forms of active oxygen. Although histamine release is affected by some of these agents, the effects on protein crosslinking may still be determined. In this way, we may learn whether the phototoxic effect of protoporphyrin is mediated directly or via active oxygen in the mast cell. The membrane stabilizing effects will also be studied in erythrocytes exposed under similar light conditions. This will determine whether erythrocytes also undergo a dual reaction to protoporphyrin and light.

In vivo studies will also be done which will determine the effects of porphyrins on mast cells. Protoporphyrin will be administered to rats in a manner known to cause phototoxic reactions. The mast cells will be removed from these animals and examined for morphological differences, viability and responsivity to histamine liberators. This project may provide information regarding the role of the mast cell in the phototoxic response. This project has been completed.

PUBLICATIONS

Ortner, M.J. and Paschall, C.S.: A circular dichroism study of protoporphyrin phototoxicity in erythrocyte ghosts. Photobiochem. Photobiophys. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50060-03 LEB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Microwave Interactions with Cells and Cellular Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Cynthia Hall	Graduate Student	N.C. State University	
	Melvyn Lieberman	Consultant	Physiology, Duke University	
	J. Paul Thaxton	Consultant	N.C. State University	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIEH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	0.6	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and subcellular level, to observe any effects of this interaction, and to relate the amount of microwave energy absorbed to the effect. The biological specimens include mitochondria cardiac cells, lysosomes, and cellular enzymes. For these experiments, a waveguide exposure apparatus has been used which can maintain the specimens at physiologic temperatures (37°C ± 0.25°C) at specific absorption rates up to 100 mW/g. No effects were noted on in vitro activity of creatine phosphokinase and acetylcholinesterase at SAR's up to 50 mW/g. Embryonic cardiac cells obtained from 9-day old quail, exhibited an increase in cell membrane permeability to trypan blue at SAR's of 10, 50 and 100 mW/g, following 90 minutes exposure. Cellular damage was noted at an SAR of 100 mW/g. Methods for examining the influence of microwave radiation on mitochondria have been developed. Preliminary results indicate the respiratory control ratio of mitochondria may be altered by in vitro microwave exposure (100 mW/g).

PROJECT DESCRIPTION

METHODS EMPLOYED: Cells and cellular components were exposed to 2450 MHz microwave radiation using a waveguide exposure apparatus developed in this laboratory. Specimens were exposed to SAR's of 1, 10, 50 and 100 mW/g for up to 90 minutes. The samples exposed include mast cells (see Z01 ES 50039-02 LEB) cardiac cells, lysosomes, mitochondria and enzymes. For each specimen certain parameters were monitored to determine the effects of microwaves on the biological specimen. In addition, when appropriate, election microscopy techniques were employed to determine microwave effects.

MAJOR FINDINGS AND PROPOSED COURSE: a. Cardiac cells. Isolated cardiac muscle cells were exposed to microwave radiation in a temperature controlled waveguide apparatus. Microwave radiation for 90 minutes at specific absorption rates (SAR) as low as 10 mW/g increases the permeability of cardiac cells to trypan blue. At 100 mW/g the inability of the cells to exclude trypan blue is concurrent with the release of lactic dehydrogenase into the suspending medium. However, when the SAR is decreased to 50 mW/g trypan blue uptake is increased without release of lactic dehydrogenase. Transmission electron micrographs of the exposed cells showed cellular damage only at the 100 mW/g exposure level. The effect on trypan blue permeability was unrelated to the macroscopic heating effect of microwave radiation on the cells, but may be due to some other specific action of microwave radiation on isolated cardiac cells. These studies have been extended to include other cell types such as macrophages, and spermatocytes (see Z01 ES 50057-02).

b. Lysosomes. At specific absorption rates of 10, 50 and 100 mW/g for 90 min at 37°C, no effects were noted on lysosomal fragility as determined by the release of the lysosomal enzymes, cathepsin D and β-glucuronidase. Furthermore, microwave exposure of the lysosomal suspension adjusted to pH 5.0 or treated with retinal, had no effect on lysosomal enzyme release. The data from this study demonstrates that microwave radiation has no labilizing effect on lysosomal membranes although other microwave-membrane interactions not associated with enzyme release may occur. These studies will be extended to include other aspects of lysosome physiology such as the response to drugs, and toxic compounds.

c. Mitochondria. Preliminary data indicated that state II, III, and IV and respiratory control rates of mitochondria exposed to microwave radiation of 100 mW/g are altered. The nature and reproductibility of this microwave effects is being examined. These studies will be extended to include endotoxin effects on mitochondria exposed to microwaves.

d. Enzymes. The effect of 2.45 GHz microwave radiation on the in vitro activity of acetylcholinesterase and creatine phosphokinase was examined. The enzyme activities were determined during exposure to microwave radiation at specific absorption rates of 1, 10, 50 and 100 mW/g. These specific absorption rates (SAR) had no effect on the activity of either enzyme when the temperatures of the control and exposed sample mixtures were similar. These data demonstrate that the activity of these two enzymes is not affected by microwave radiation at the SAR's and frequency employed in this study.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment are of interest to the National Institute of Environmental Health Sciences. Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control the temperature of the specimen carefully and be able to reproduce the exposure conditions. We have developed the capability to do this for in vitro exposures at NIEHS and this may provide a system for differentiating specific microwave effects from thermal responses. In addition, by examining the response of cells and cellular components to microwave radiation, it should be easier to identify the mechanism of action of microwave radiation with biological specimens.

PUBLICATIONS

Galvin, M.J., C.A. Hall, and D.I. McRee: Microwave radiation effects on cardiac muscle cells in vitro. Rad. Res., 86: 358-367, 1981.

Galvin, M.J., D.L. Parks and D.I. McRee: Influence of 2.45 GHz Microwave Radiation on Enzyme activity. Rad. Environ. Biophys. 19: 149-156, 1981.

McRee, D.I., M.J. Galvin, C.A. Hall, and M. Lieberman: Microwave Effects on Embryonic Cardiac Tissue of Japanese Quail. Berteaud, A. and Servanitie, B. (Eds.): Proceedings of International Symposium on Electromagnetic Waves & Biology, Paris, France, pp. 79-84, 1980.

Galvin, M.J., D.P. Parks, G. MacNichols and D.I. McRee: Influence of microwave radiation on in vitro release of enzymes from retinol treated hepatic lysosomes. Cell Biophysics. 3: 175-186, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50062-03 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Enzymatic Reduction of C-Nitroso Compound

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
	Volker Fischer	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The reduction of C-nitroso compounds such as nitrosobenzene and 2-methyl-2-nitrosopropane to nitroxide free radicals will be investigated. Although nitroso compounds are important reduction products of the more numerous nitro compounds, the enzymatic reduction of these compounds has received little attention. The biochemical implications of the reactions of the nitroxide intermediates will also be investigated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The enzymatic reduction of 2-methyl-2-nitrosopropane results in a four-line spectrum due to t-butyl hydronitroxide. The concentration of this free radical increased for over 30 min. This free radical accumulated in the presence of catalase (30,000 units/ml), but not in the presence of superoxide dismutase (30 μg/ml). Inhibition by superoxide dismutase is consistent with superoxide oxidation of the t-butyl hydroxylamine reduction product or reduction of the 2-methyl-2-nitrosopropane by superoxide.

Nitrosobenzene is known to be reduced by ascorbate to a species which will reduce ferricytochrome c. Other investigators have proposed this species to be an oxygen-reactive hydroxylamine radical R-NOH.

$$R\text{-}N = O + AH \longrightarrow R\text{-}\dot{N}\text{-}OH + A^{\cdot}$$

$$R\text{-}\dot{N}\text{-}OH + O_2 \longrightarrow R\text{-}N = O + O_2^{\cdot-}$$

A nitroxide free radical is more likely to be the species formed as can be confirmed by ESR.

$$R\text{-}N = O + AH^{-} \longrightarrow R\text{-}\overset{O^{\cdot}}{N}H + A^{\cdot}$$

The reaction of a hydronitroxide with oxygen to reform the parent nitroso compound and superoxide is a possible, but unlikely, reaction, because such nitroxides are easily observed in the presence of oxygen. On the other hand, it is well known that ascorbate can reduce nitroxides to form hydroxylamines and the air oxidation of hydroxylamines to reform nitroxides is known to occur. These two reactions could account for the reported oxygen uptake in the presence of nitrosobenzene and ascorbate.

$$AH^{-} + R\text{-}\overset{O^{\cdot}}{N}H \longrightarrow AH^{\cdot} + R\text{-}\overset{H}{\underset{O}{N}}$$

$$R\text{-}\overset{H}{\underset{O}{N}} + O_2 \longrightarrow R\text{-}\overset{O^{\cdot}}{N}H + O_2^{\cdot-}$$

Although it is well known that ascorbate can reduce nitroxides to form hydroxylamines, the reduction of nitroso compounds by either cofactors or enzymes to form hydronitroxides has not been reported.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 4-Nitroso-quinoline-N-oxide, 2-nitrosoflurene and 2-nitroso-2-naphthanol are a few of the nitroso compounds proposed to be ultimate carcinogens derived from the corresponding nitro compounds. Although the nitroxides are probably not DNA alkylating agents, they are probably intermediates in the formation of such species.

PUBLICATIONS

Mottley, C., Kalyanaraman, B. and Mason, R.P.: Spin trapping artifacts due to the reduction of nitroso spin traps. FEBS Letters 130: 12-14, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50063-02

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological Effects of High Pressure Sodium Vapor Lamps

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Colin F. Chignell Chief LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sprague Dawley rats were born and reared under either daylight-simulating fluorescent lights or high pressure sodium vapor (HPSV) lamps. The illuminances of the two lighting environments were adjusted so that the perceived brightness was the same. Rats housed under the HPSV lamps had heavier adrenals, smaller gonads (males only), larger kidneys (females only) and elevated red and white cell counts. No differences were observed between the two groups in the swim endurance, tail flick and hotplate tests.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purpose of this study is to determine the effects of high pressure sodium vapor (HPSV) lamps on the development of the Sprague Dawley rat. Adult Sprague Dawley rats were paired and housed under either daylight simulating fluorescent lights or HPSV. The brightness of the two environments was normalized by adjusting the illuminance of the daylight lights to 10 ft. candles (48 $\mu W/cm^2$) while the illuminance of the HSPV lamps was kept at 30 ft. candles (140 $\mu W/cm^2$). The pregnant females were allowed to give birth and the litters were normalized to four males and four females soon after birth. The effect of the two lighting conditions on the following parameters was assessed: organ weights, peripheral hematology, swim endurance, tail flick and hotplate tests.

MAJOR FINDINGS AND PROPOSED COURSE: The weight of the adrenals was signifcantly higher ($p<0.001$) in both male and female rats housed under the HPSV lamps. In addition the male rats had smaller testes (373 mg/100 g body weight vs 394 mg/100 g body weight), and the females had larger kidneys (705 mg/100 g vs 680 mg/100 g) when exposed to the HPSV lamps. The HPSV lamps also caused an elevation in red blood cell count (males and females) and an elevated white cel' count (males only). No difference were observed between the two groups in the swim endurance, tail flick and hotplate tests.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although high pressure sodium vapor lamps are more efficient than conventional incandescent lights their energy spectrum is considerably different from that of natural daylight. Since high pressure sodium vapor lamps are now being used more extensively for lighting purposes in school and offices it is important to determine whether they produce any undesirable biological effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50064-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Behavorial Effects of Noise Exposure During Fetal Life

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hugh Tilson	Pharmacologist	LBT	NIEHS
	Reginald Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics/Lab. of Behavioral & Neurological Tox

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Noise stress during pregnancy has been reported to be embryolethal, as well as teratogenic, in rats and mice. Although there have been few studies concerning the possible effects of prenatal noise stress on the development of neurobehavioral functioning of the offspring, it is known that a combination of light and heat stress has been reported to cause changes in catecholamine levels in brain areas affecting sexual differentiation of adult rats exposed as fetuses and to cause demasculinization of male offspring. The purpose of this experiment is to determine the effects of prenatal noise stress on selected tests of neurological functioning and reactivity. The results of these experiments might be used in the planning of future studies that (1) compare the effects of noise stress to other stressers (2) measure those changes in neurochemical and neuroendocrinological status in stress exposed animals that may be correlated with any behavioral changes.

PROJECT DESCRIPTION

METHODS EMPLOYED: The subjects used in this study were obtained from female albino rats of the Fischer 344 strain (Harlan Industries, Indianopolis, Ind.) on day 5, sperm positive females were housed in single stainless steel wire cages (25 + 35 x 22 cm) contained within sound and light attenuating cubicles (IAC 400) equipped with ventilation fan, temperature control and automatic lighting. Lights were turned on at 0600 hrs and turned off at 1800 hrs. Food (NIH lab chow #31) and water were freely available.

Rats were randomly selected to receive exposure to noise on days 6 through 21 of gestation. For 12 hours daily (1200 to 2400 hrs), one-half of the rats were exposed to an octave band of noise (amplitude 110 dB, linear) centered on the region of most acute auditory sensitivity (8 kHz), while the remaining animals were housed under similar condition, but exposed to ambient levels of noise (≈20-30 db inside the cubicle).

The dams were weighed on days 5 and 19 of gestation. On the day of littering, the birth weights and litter size of the offspring, were recorded and the mother and pups removed from the acoustic chambers and housed in our standard animal housing quarters. Because of the relatively high variability in litter size, the litters were culled to 3-4 pups per litter. The sex distribution within the litter was equalized to the extent possible.

The pups remained with their natural mother until 21 days of age, at which time they were weaned. One male and one female were randomly selected from each litter for subsequent behavioral testing at 30 and 100 days of age. Rats selected for future study were housed by treatment in unisex groups of four.

At 30 and 100 days of age, the rats were weighed and then assessed by a standard battery of neurobehavioral tests. Briefly, fore- and hind limb grip strength were measured using commercially available strain gauges. Startle responsiveness was measured by placing the animals on a platform attached to a load cell and preventing them with a 90 db, 4 msec solenoid closure sound stimulus. Responses to an air puff stimulus delivered by an air bulb device were measured following preventation of the acoustic stimulus. Reactively to a noxious stimulus was determined by a standard tail flick analgena away. Spontaneous motor activity occurring over a 60 min period was measured using automated motor activity monitors. Swim endurance was measured by placing the rats in a round vessel containing water at approximately, 20°C. A small weight (lead fishing weight) amounting to 10% of the animals body weight was affixed to the tail. The latency required to observe complete submergence for at least 5 sec was recorded as the swim endurance measure. All tests were done "blind".

MAJOR FINDINGS AND PROPOSED COURSE: Repeated measures ANOVA indicated that gestational exposure to noise did not affect body weights when measured at 30 and 100 days ($F=0.50$) body weights did increase as a function of age ($F=4113,89$; $df=1,28$; $p<0.0001$); males weighed more than females ($F=386.49$; $df= 1,28$; $p<0.001$). The most robust effect of gestational exposure to noise appeared to be on measures of neuromotor function. The forelimb grip strength of noise exposed pups was significantly affected ($F=4.952$; $df=1,28$; $p<0.05$). There was a significant

treatment by sex interaction ($F=10.72$; $df=1,28$; $p<.0001$) and subsequent post hoc comparisions with Fisher's least significant difference tests showed that noise-exposed females had lower grip strength at 100 days of age than controls. Exposure to noise during gestation also effected hind limb grip strength ($F=7.48$; $df=1,28$; $p<0.025$). Both males and females exposed to noise during gestation had significantly lower hind limbs grip scores at 100 days of age. Swim endurance, another measure of neuromotor capacity was also affected by noise ($F=6.56$; $df=1,28$; $p<0.025$). However, unlike grip strength, swim endurance was affected most at 30 days of age, rather than at 100 days of age. Swim endurance of both males and females was affected by exposure to noise. The effects of noise exposure on neuromotor function appeared relatively specific to measures of strength or endurance; exploratory activity occurring in 60 min test periods was not affected by noise exposure ($F=0.09$).

Gestational exposure to noise had few, if any effects on reactivity to noxious and non-noxious stimuli. Tail flick latencies were not significantly affected nor were response to air puff and acoustic stimuli ($F=0.48$).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Accumulating evidence indicates that exposure of rodents to elevated noise levels during pregnancy usually leads to decreased pregnancy maintainence and occasionally to reduced fetal weights, fetotoxicity or teratogenesis. Since both plasma and uterine catecholamine and to a lesser degree, corticosterone levels have been found elevated by noise exposure in late stage pregnancy, it is possible that these findings of decreased neuromotor function, are somehow related. The absence of control-exposed differences in most of the neurobehavioral tests employed suggests that the effects of noise stress may be very specific. Sexual competence in male offspring has been found to be a consistent correlate of maternal stress during pregnancy in experients utilizing other physical stressors (restraint, blinking lights); this end point should be similarily examined for noise stress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50065-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Superoxide and Lipid Peroxidation in Biological Properties of Anthracyclines and DHAQ

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Birandra K. Sinha Senior Staff Fellow LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemical properties (antitumor, carcinogenic and cardiotoxicity) of the anthracyclines may be related to DNA binding and or the production of oxygen derived toxic free radical metabolites. We have examined the formation of superoxide ($O_2^{\cdot-}$) from these drugs and have evaluated the role of oxygen derived toxic species in their biological properties. Our findings indicate that formation of $O_2^{\cdot-}$ is not related to the antitumor activity. However, lipid peroxidation caused by $O_2^{\cdot-}$ or $OH^{\cdot}$ is related to the cardiotoxicity of the anthracyclines.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal-NADPH incubations of both adriamycin and daunomycin have been shown to generate semiquinone free radical intermediates which react with oxygen to form superoxide ($O_2^{\cdot-}$). The semiquinone free radical or $O_2^{\cdot-}$ or its toxic metabolites (OH/H_2O_2) have been recently proposed to act as primary cytotoxic agents. In addition, $O_2^{\cdot-}$ and OH/H_2O_2 have been implicated in anthracycline-induced cardiotoxicity through lipid peroxidation of the cardiac cell membranes.

MAJOR FINDINGS AND PROPOSED COURSE: The ability of six anthracyclines and 1,4-bis-{2-[(2-hydroxyethyl)amino]ethylamino}-anthracenedione (DHAQ), with demonstrated antitumor activity and cardiotoxicity, to stimulate $O_2^{\cdot-}$ production in rat hepatic microsomes and beef heart submitochondrial particles has been studied. Our findings indicate that the semiquinone free radical is formed in heart submitochrondrial particles which stimulates the production of $O_2^{\cdot-}$ in a manner similar to that seen with rat hepatic microsomes. While daunomycin stimulated a large production of $O_2^{\cdot-}$ in rat hepatic microsomes, the most active antitumor agents, DHAQ and AD-32, stimulated significantly smaller production of $O_2^{\cdot-}$. This would suggest that the antitumor activity may not be related to $O_2^{\cdot-}$ formation. In addition, fully intercalated drugs are not effective in $O_2^{\cdot-}$ production and hence cannot act as carriers for site specific production of $O_2^{\cdot-}$.

Our studies also show that these agents induce peroxidation of rat hepatic microsomal lipid. Agents which are highly cardiotoxic (adriamycin, daunomycin and N, N-dimethyl daunomycin) induce significantly more lipid peroxidation than those agents which are less cardiotoxic (AD-32, rubidazone, carminomycin and DHAQ). Thus it appears that lipid peroxidation plays an important role in the pathogenesis of cardiotoxicity. Furthermore, α-tocopherol, a known free radical scavenger, afforded only a marginal protection against lipid peroxidation induced by these agents. Reduced glutathione, on the other hand, afforded a complete protection against lipid peroxidation. This project is essentially complete and no other work is planned at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since oxygen derived toxic free radical metabolites have been implicated in genesis, cytotoxicity, and cardiotoxicity of a number of chemicals it is of great interest to understand the molecular mechanism of $O_2^{\cdot-}$ ($OH;H_2O$) formation and their relevance in the expression of toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50066-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Reactions of Free Radicals from Serotonin and Related Indoles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
OTHERS:	Hartmut Stegman	Guest Worker	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ESR spectra of the free radicals formed by the autoxidation of serotonin, 5-hydroxyindole, and 5-hydroxytryptophan in 1 N NaOH have been detected. The analysis of the hyperfine splitting constants in H_2O and D_2O characterize these free radicals as semiquinone-iminies, the one-electron oxidation product of the corresponding indole. At alkaline pH, autoxidation of these compounds ultimately leads to solid precipitate and unresolved ESR spectra characteristic of polymeric material. The reduction of cytochrome *c* at pH 7.4 by a wide variety of indoles correlates with the amplitude of the ESR signal in 1 N NaOH, as do other processes though to be related to 5-hydroxyindole free radical formation. Relative to the rate of cytochrome *c* reduction, neither serotonin nor the serotonin free radical appears to react with oxygen to form superoxide. In the presence of NAD(P)H, the serotonin radical most probably oxidizes NAD(P)H to form the NAD(P)· radical. The NAD(P)· radical then reacts with oxygen to form superoxide, which ultimately reduces cytochrome *c*. This work has been extended to the dihydroxytryptophans, which are neurotoxins.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: A serotonin free radical was observed by Borg in 1964 using electron spin resonance. The radical was generated during permanganate oxidation in alkaline solution ($KMnO_4$, 0.1 N NaOH); however, the biphasic decay kinetics suggested the presence of two free radical species. Due to the importance of serotonin, we decided to reinvestigate the ESR spectrum of its free radical. In addition, we have studied the ESR spectra of 5-hydroxytryptophan and 5-hydroxyindole using deuterium isotope substitution and, where possible, an ESR resolution enhancement technique of 90° out-of-phase dection.

The history of the serotonin radical begins in 1961 when Walaas and Walaas presented spectrophotometric evidence that ceruloplasmin could generate the radical, which then oxidized reduced pyridine nucleotides. Later, Alivisatos and Williams-Ashman reported the synergistic stimulation of cytochrome *c* reduction by serotonin and NADH. The mechanism of this stimulation was further studied under anaerobic conditions by Polacow and Cilento. They postulated that serotonin made cytochrome *c* more susceptible to reduction by NADH, without the formation of the serotonin radical. We have reinvestigated this system under aerobic conditions and report the effect of superoxide dismutase and catalase. Our ESR and cytochrome *c* reduction results demonstrate the importance of free radical reactions of serotonin and other 5-hydroxyindoles.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Free radical reactions of the serotonin semiquinone-imine free radical with itself, serotonin, serotonin quinone-imine, and protein may be important in the formation of this melanin-like material. Whether 5-hydroxyindole-derived melanin actually forms in the brain is unknown, but *in vitro* experiments clearly indicate that the one-electron oxidation of serotonin is possible under physiological conditions. The autooxidation of dihydroxytryptophans is thought responsible for the neurotoxicity of these compounds. The semiquinone free radical is the first intermediate formed by autooxidation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50067-02 LEB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular Studies of Anthracene Phototoxicity in Rat Mast Cells and Human Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Anthracene, a component of coal tar, has proven to be a very potent photosensitizer in human erythrocyte ghosts. Anthracene was bound covalently to different positions on fatty acid molecules. Phototoxicity studies showed that when anthracene was bound to the polar end, it was more photoxic than anthracene alone. However when bound to the apolar end, anthracene had little phototoxic effect. The major site of anthracene phototoxic reactions therefore appear to be close to the protein-lipid interface within the ghost membranes.

PROJECT DESCRIPTION

OBJECTIVES: The light-excited triplet state of endogenous photosensitizers (e.g. protoporphyrin) or phototoxic drugs (e.g. tetracycline) initiates oxidative reactions within erythrocyte membranes that ultimately lead to membrane damage and hemolysis. The most damaging molecular reaction is probably intermolecular protein crosslinking caused by covalent bond formation between free amine groups and photooxidized histidine residues. Although the generation of active oxygen (e.g. O_2, $^1O_2$) has been implicated as an intermediary in this reaction, the primary molecular mechanism of phototoxic reactions is unknown.

Anthracene, a polycyclic aromatic hydrocarbon component of coal tar, is currently being studied. This environmental pollutant is a potent photosensitizer and may prove useful in determining the molecular mechanism of light-induced toxic reactions.

METHODS EMPLOYED: Human erythrocytes are being used to study the phototoxic effects of anthracene on cell lysis and on isolated membrane proteins. In addition, rat peritoneal mast cells are being used to study phototoxic reactions in a functioning secretory cell. Standard illumination techniques are employed and circular dichroism spectroscopy has also been developed with the objective of identifying the molecular species directly involved in protein photodegradation.

MAJOR FINDINGS AND PROPOSED COURSE: Erythrocytes exposed to anthracene show up to 78% hemolysis after 90 min illumination. The effect is concentration and time dependent and does not occur in the dark. Recent experiments using anthracene-derivatized fatty acids have shown that anthracene is much more toxic to erythrocytes when covalently bound close to the polar end of the fatty acid molecule (2-anthroyloxy-palmitic acid). Anthracene attached to the 16th carbon of palmitic acid was not effective as a photosensitizer. These data indicate that the site of photosensitization by anthracene is near the interface region of the erythrocyte membrane. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Coal tar derivatives constitute a significant contribution to environmental pollution. It is important therefore to understand the mechanism of anthracene toxicity, especially in relation to a possible synergistic effect with sunlight. The present study is currently evaluating the effects of anthracene in both erythrocytes and secretory cells. Since very little is known about the phototoxic effects of this compound, we are also studying its molecular mechanism and its site of action in the membrane. These studies may also prove useful in the clarification of phototoxic mechanisms in general.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50068-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Acute in vivo Exposure of Rats to 2450 MHz Microwave Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute 8 hour exposure of rats to 2450 MHz (CW) microwave radiation was performed at power levels that cause no increase in colonic temperature. There were no measurable changes in the mast cell response to compound 48/80 or to concanavalin A, nor were any morphological changes observed. There was no change in the thyroid axis, however alterations were seen in serum corticosterone levels in the 10 mW/g (SAR) group. No change in hematocrits or erythrocyte number was observed and there were no changes in total leukocytes, or in the numbers of lymphocytes, neutrophils, and monocytes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Acute exposure to 2450 MHz microwaves radiation in the rat has been studied previously for varying periods of time. Although high power densities cause significant biological changes, these alterations are usually accompanied by substantial increases in colonic temperature. However, several studies have reported microwave-induced changes in immunocompetence, hematopoiesis, cytological parameters and neuroendocrine secretion at incident power densities of 20 mW/cm^2 or less. Under these conditions, there was no apparent temperature increase and the biological effects have been attributed to other putative microwave characteristics.

We have studied the effects of acute exposure at two power densities on several systems in the rat. Peritoneal mast cells store several potent vasoactive agents (e.g. histamine). Membrane activation through several distinct pathways can cause active secretion of these factors and their consequent physiological effects. Mast cells are thus responsive to a wide variety of external stimuli and their secretory ability is influenced by several biochemical and physiological parameters. The possibility therefore exists to demonstrate both primary and secondary microwave effects in vitro.

The mammalian hypothalamo-hypophyseal-thyroid (HHT) axis and hypothalamo-hypophyseal-adrenal (HHA) axis of the endocrine system regulate body functions that maintain homeostasis in basal and stress states. The functional competence of these axes is essential to survival of the animal within a changing environment. Since microwave exposure can be considered as a type of environmental stress, its effects on the activities of the HHT and HHA axes are important.

Hematologic and biochemical effects of microwave radiation have been reported including a significant leukopenia, lymphopenia and neutrophilia, and significant increases in blood glucose, cholesterol and glutamic oxaloacetic transferase activity. These data suggest that blood composition (hematology and biochemistry) is sensitive to microwave radiation.

METHODS EMPLOYED: Exposures were carried out within an environmental chamber. Both control and exposed animals were housed individually in styrofoam cages. The animals were in positions which received similar field intensities. The incident power density was determined directly using NBS Model B probe inserted in a sample cage with and without animals in the field. Each experimental group consisted of 8 male rats (Charles River, CD) with weights of 300 $\pm$ 35 g. Twelve experiments (4 control, 4 low and 4 high power exposures) were run on consecutive days alternating the control, low power density (2 mW/cm^2) and high power density (10 mW/cm^2) groups. The control rats were treated exactly as the exposed ones except that the power was not turned on. The animals were weighed immediately before and after exposure. The animals were irradiated for 8 hrs with food and water withheld to prevent aberrations in the field. Within 5-15 min after irradiation, six animals were decapitated, the peritoneal mast cells extracted in Lockes solution, and blood was removed for hematology and endocrinology determinations. The two remaining animals were immediately anesthetized with pentobarbital and the aorta cannulated for blood pressure determinations.

MAJOR FINDINGS AND PROPOSED COURSE: The data on wave penetration in biological tissue indicate that under our exposed conditions, the blood basophils, the cutaneous mast cells and probably most of the peritoneal mast cells received direct microwave exposure. In addition, some conductive heating probably continued beyond the actual depth of penetration (approximately 2 cm). Nevertheless, the viability studies showed that this potential stressor did not greatly damage the cells of the peritoneal cavity or disturb the normal basophil count in the blood.

We have studied the performance of irradiated mast cells stimulated via the chemical (48/80) and the immunological (Con A) pathways. Compound 48/80 is one of the most potent and efficacious histamine liberators and it acts by stimulating putative receptors on the cell membrane. Con A, in contrast, stimulates the cell by crosslinking membrane-bound IgE molecules in a manner similar to antigen-antibody interactions. The proper functioning of both pathways depends on cellular energy metabolism, cyclic nucleotides, cytoskelletal elements and in the case of Con A, calcium transport. The present in vivo irradiation data not confirm our previous findings in in vitro which showed that microwave radiation did not affect the complicated secretory pathways of these activators. Furthermore, we have shown that after intraveous injection of 48/80, the in vivo secretory response of the basophils and mast cells was unimpaired. This indicates that these cells are not adversely affected by any secondary factors potentially brought about by the radiation (e.g. release of hormones, neurotransmitters, etc.).

Serum concentrations of T_4 and T_3 were not altered by the experimental conditions used. T_3-uptake values, representing the unsaturated binding capacity of thyroid binding globulin, were similarly unaffected. FTI and AT_4, values, which are calculated from the T_3-uptake data and are proportional to free T_4 levels, did not exhibit significant differences between treatment groups. Rats exposed for 8 hours to 0 mW/cm^2 or 2 mW/cm^2 had higher serum corticosterone levels than did untreated control rats. Those exposed to 10 mW/cm^2 did not exhibit an increase as did the 0 and 2 mW/cm^2 groups, but rather had values similar to the untreated controls.

The level serum protein in general and of β-glucuronidase, alkaline phosphatase, catic dehydrogenase and cholinesterase in particular was not altered by microwave exposure at either power density. In addition, no effects of microwave radiation were noted on sodium and potassium levels. No change in the hematocrit or RBC number was noted for either exposure group, furthermore, there were no changes in the total number of circulating white blood cells, or in the precentage of white cell types, including lymphocytes, neutrophils, or monocytes.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent advances in microwave technology have resulted in increased potential for exposure of both the general public and technical personnel to non-ionizing radiation. Because the biological consequences of acute exposure to alternating electromagnetic fields are largely undefined, investigations utilizing microwave radiation are of current interest. The present studies have shown that acute exposure to microwave radiation does have significant effects on certain endocrine parameters, whereas other systems are unaffected. The results suggest that there are

target systems that are especially sensitive to microwave induced stress. This project has been completed.

PUBLICATIONS

Ortner, M.J., M.J. Galvin and D.I. McRee: Studies on acute in vivo exposure of rats to 2450 MHz microwave radiation. I. Mast Cells and Basophils. Rad. Res. 86: 580-588, 1981.

Abhold, R.H., M.J. Ortner, M.J. Galvin and D.I. McRee: Studies on acute in vivo exposure of rats to 2450 MHz microwave radiation II. Effects on thyroid and adrenal axis hormones. Rad. Res. 88: 448-455, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50069-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biophysical Studies on the Effects of 2450 MHz Microwave Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Richard Irwin	Chemical Manager	TRTP	NIEHS

COOPERATING UNITS (if any)

Toxicology Research and Testing Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Instrumentation has been developed for the study of fluorescence or circular dichroism during exposure of biological samples to microwave radiation (2450 MHz, CW). Fluorescence experiments have shown that microwave radiation at SAR's of 10 and 200 mW/g had no effect on calcium binding to human erythrocyte membranes or on energy transfer between membrane bound probes and intrinsic tryptophan residues. Circular dichroism measurments have shown that spectrin molecules from human erythrocyte membranes may be affected by high microwave power levels (600 mW/g, SAR). These effects may result from differential intramolecular interactions with the oscillating electric field. Experiments utilizing these instruments will help to clarify reported microwave effects by examining them on a molecular basis. The studies have been extended to include the effects of microwave radiation on microtubular polymerization in vitro.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Microwave induced alterations in animal physiology must eventually be defined in terms of cellular pathophysiology and ultimately on a molecular level. We have therefore developed two biophysical instruments that interface precisely with sample chambers to provide accurate microwave exposure, dosimetry and temperature control. Molecular studies of drug binding and membrane protein conformation can thus be conducted before, during and after exposure of a sample to microwave radiation.

METHODS EMPLOYED: The effect of 2450 MHz microwave radiation on the proteins of human erythrocyte ghosts has been investigated using ultraviolet circular dichroism spectroscopy. A specially constructed waveguide inserted into a spectropolarimeter allowed continuous recording of the optical activity due to secondary structure in membrane proteins. Microwave-induced conformational changes in protein α-helical structure could then be compared with the effects of conventional heating.

The effect of 2450 MHz microwave radiation on erythrocyte membrane protein conformation and calcium binding was studied with the fluorescent probe, 1-anilino-8-naphthalenesulfonate (ANS). Using fiber optic cables, excitation light was delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a fluid-filled, temperature-controlled waveguide. Fluorescence was collected using an identical cable and transferred through appropriate filters to standard detecting, amplificaton and recording devices.

MAJOR FINDINGS AND PROPOSED COURSE: The ultraviolet circular dichroism data indicate that high levels of microwave radiation (600 mW/g, specific absorption rate) induce decreases in α-helical conformation that may be due both to thermal vibrations and to increased strain on the intramolecular hydrogen bonds that maintain secondary structure. The latter effect may result from differential intramolecular interactions with the oscillating electric field. Spectrin (bands 1 and 2) isolated from the ghosts was more sensitive to microwave irradition than intact ghosts, and spectrin-depleted vesicles were the least sensitive. The data, therefore, indicate that the α-helical conformation of spectrin is altered by high levels of microwave radiation.

Microwave radiation at specific absorption rates of 10 and 200 mW/g had no effect on the binding of the fluorescent probe, ANS, to the erythrocyte membranes. Concentration response curves of increased fluorescence intensity versus calcium concentration also showed no microwave influence on calcium binding between 2.0 - 10.0 $\times 10^{-4}$M. In addition, experments studying fluorescence energy transfer between intrinsic tryptophan residues and membrane bound ANS showed that intermolecular distances between donor and acceptor were also unaffected by microwave radiation.

These specially developed instruments are being used to investigate possible molecular mechanisms responsisble for the many putative effects of microwave radiation, including possible synergistic effects. Both circular dichroism and the fluorescence detecting instrumentation are now being used to study effects of simultaneous microwave exposure on the polymerization of microtubules and specific drug binding (e.g. colchicine, vinblastine) to tublin.

The effect of microwave radiation on brain mitochondrial membranes is also being investigated using ANS, which is sensitive to changes in charge distribution due to substrate (e.g succinate, NADH) oxidations. Because of the evidence supporting a specific microwave effect on embryogeny, circular dichroism spectroscopy will be used to study microwave effects on both intact chromatin and isolated DNA. Chromatin will be studied using intact avian erythrocyte nuclei. Circular dichroism will also be useful to study the effects of microwaves on drug binding to DNA, cell membranes and subcellular organelles. Future fluorescence projects include a study of the reported synergism between chlordiazepoxide and microwaves. The effects of irradiation during the binding of this and other neuropharmacological drugs to isolated brain synaptosomal membranes will be studied. Microwave effects on fertility have been demonstrated in this laboratory after irradiation of avian sperm. The effect of microwaves on sperm membrane protein sulfhydryl groups and sperm-tail microtubular protein will therefore also be studied using fluorescent probes. This project is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent advances in microwave technology have resulted in increased potential for exposure of both the general public and technical personnel to non-ionizing radiation. Because the biological consequences of acute or chronic exposure to alternating electromagnetic fields are largely underfined, investigations utilizing microwave radiation are of current interest. Teratological, neurological, immunological and hematological alterations in both avian and mammalian species have been ascribed to a putative "non-thermal" or "electromagnetic" stress following whole body exposure to microwave radiation. In addition, recent reports have suggested that low level exposure to microwaves may also sensitize animals to the effects of drugs in a synergistic manner, presumably by inducing an occult stress. Unfortunately, the data are often contradictory due to a wide variation in experimental techniques, frequencies and animal models and the subtle nature of the putative effects. As a result, a unifying mechanistic concept to explain the biological effects of microwave radiation has not been developed.

In order to understand the biological effects of microwave radiation, the - alterations in normal physiology must eventually be defined in terms of cellular pathophysiology and ultimately at the molecular level. The development of the present methodology will aid in explaining many of the putative effects of microwave radiation.

PUBLICATIONS

Ortner, M.J., M.J. Galvin, C.F. Chignell and D.I. McRee: A circular dichroism study of human erythrocyte ghost proteins during exposure to 2450 MHz microwave radiation. Cell Biophysics, 3, 335-347, 1981.

Ortner, M.J., M.J. Galvin, D.I. McRee and C.F. Chignell: A novel method for the study of fluorescent probes in biological material during exposure to microwave radiation. J. Biochem. Biophys. Meth. 5: 157-167, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50070-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of Different Noise Exposures on Embryo/fetal Development in the Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
OTHER:	Peter S. Nawrot	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Z01 ES 50045-04 LEB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50071-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Electro Acoustic Factors Affecting Speech Discrimination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS
OTHER:	W.G. Thomas	Director, Hearing and Speech Program	N.C. Memorial Hospital
	Blake Wilson	Senior Electrical Engineer	RTI

COOPERATING UNITS (if any)

N.C. Memorial Hospital and RTI

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A line of investigation which began with the limited purpose of comparing the discriminability of speech mechanically coupled to the ossicular chain with similar speech passed through hearing aids, led to an awareness that the manner in which speech discrimination formulations were conventionally utilized failed to take into account important electro acoustic variables. Pilot tests suggested that failure to account for these variables might be a prime factor in the continuing controversy regarding the precision of phonetically balanced monosyllablic word lists, the most widely used speech discrimination formulation. Attempts to systematically elucidate the relevant parameters were initially focused on the method of "equalizing" speech made dissimilar in speech peak to long term RMS level by amplitude distortion. Discrimination scores obtained by listeners from lists which had been equalized on the basis of their long term RMS level showed that this method was superior to conventional peak equalization methodologies in preserving the intrinsic discrimination superiority of less distorted speech.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electronic measurement systems were utilized to precisely assess, analyze, and compare electro-acoustic parameters of conventional speech formulations which had been deliberately made unequal by passage through differentially distorting transducers. Listener test scores were obtained to determine the intelligibility of the resulting speech when equalized according to long term RMS level and the conventional peak method.

MAJOR FINDINGS AND PROPOSED COURSE: In discrimination tests where electro-acoustic amplitude parameters were systematically varied it was shown that confounding occured when the conventional peak equalization method was used to equalize significantly distorted speech material with nondistorted or lightly distorted speech, obviating the intrinsic discrimination superiority of the latter. When the different materials were equalized on the basis of total energy content, listener tests showed that the intrinsic discrimination superiority of the less distorted speech was preserved. By utilizing this technique it was also shown that speech mechanically coupled to the ossicular chain of guinea pigs and recovered from the inner ear by differential electrode techniques, was discriminated equally with speech which was acoustically coupled, but was discriminated significantly better than speech passed through conventional hearing aids. In addition, an electronic device which will make it possible for others to test a variety of equalization procedures was designed and fabricated.

Techniques validated as a result of the above investigations will be employed in recording new test tapes designed to (1) determined whether speech spectrum noise and babble of equal spectral density are equally efficient speech maskers, (2) determine the effect of matching signal and competition spectrum on discrimination test results, and (3) determine whether speech discrimination differences which may occur when competion and speech signal are passed through hearing aids separately and simultaneously correlate with hearing aid intermodulation distortion characteristics. The role of the Noise Effects Group will be limited to preparing the tapes, others will acquire the listeners and perform the actual testing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Scientifically validated procedures are needed for assessing the degree of handicap for speech discrimination associated with varying amounts of pure tone hearing loss and for objectively relating hearing aid characteristics to hearing loss characteristics. Without well validated procedures it is difficult, if not impossible, to assess the costs associated with the social and economic impairment suffered by our 16 million hearing impaired citizens. Without realistic hearing loss cost figures, the benefits associated with hearing conversation-noise reduction programs cannot be calculated, making cost-benefit decisions highly subjective.

PUBLICATIONS

Cook, R.O., Hamm, C.W., Thomas, W.G. and Royster, L.W.: Comparison of acoustically coupled and direct ossicular chain coupled speech. Audiology 20(6): 516-529, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50072-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ototoxicity of Cis-dichlorodiammine Platinum (II)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Bhola N. Gupta	Pathologist	TRTP	NIEHS
	Jiri Prazma	Assistant Professor	Dept. Surgery, UNC Med. School	

COOPERATING UNITS (if any)
UNC at Chapel Hill

Toxicology Research & Testing Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cis-dichlorodiammine platinum, an agent with antineoplastic activity, was examined by otototic effects in guinea pigs. Ototoxicity was evaluated by suppression of the cochlear potentials, alteration of electrolyte concentrations in the cochlear fluids and by histopathological changes in the inner ear. Guinea pigs treated with ip injection of cis-dichlorodiammine platinum 1.5 mg/kg (5 doses/wk) developed marked suppression of sound-evoked cochlear responses but showed little changes in the endocochlear potential. The electrolyte concentrations of the cochlear fluids were not substantially altered in cis-dichlorodiammine platinum-treated guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pigmented guinea pigs were used. The evaluation of hearing was performed by electrocochleography during the period of treatment with cis-dichlorodiammine platinum (II) (Cis-DDP). Under pentobarbital sodium anesthesia a silver ball electrode was chronically implanted at the retroauricular regions and a reference silver electrode was placed at the vertex. After recovery from surgery the averaged action potential (AP) in response to click stimuli were recorded under analgic condition with ketamine. The electrocochleography was carried out periodically before and during treatment periods. Following treatment guinea pigs were anesthetized with intraperitoneally injected pentobarbital sodium. The cochlear sound-induced responses and the endocochlear potential (EP) were recorded from the basal and third turns of the cochlea. Samples of the endolymph and perilymph were collected and Na^+, K^+ and Cl^- concentrations were determined. Surface preparations of the organ of Corti were studied in four cis-DDP treated guinea pigs which showed various degrees of suppression of the cochlear potentials. Kidneys of seven guinea pigs treated with Cis-DDP were studied histologically.

One group of 14 guinea pigs was injected intraperitoneally with daily doses of 1.5 mg/kg five times a week until electrocochleography showed persistent and complete suppression of AP to click stimuli at 73 dB_{peak} SPL. The second group of 4 guinea pigs was injected intraperitoneally with sterile water, 1.5 ml/kg five times a week for periods of 3 to 4 weeks. An additional 16 guinea pigs without treatment were used as controls.

MAJOR FINDINGS AND PROPOSED COURSE:

1. General conditions. Treatment with daily ip injections of 1.5 mg/kg cis-DDP five times a week did not cause mortality. The general condition of treated quinea pigs was not greatly affected throughout the experiments. Gradual loss of body weight was observed (mean loss 9 ± 2.5 g per week), during the 2nd and 3rd weeks of treatment. However the gait was normal and no vestibular disturbance was observed.

2. Electrocochleograms. The AP responses were stable during the pretreatment period. The amplitude of N_1 response to unfiltered clicks at 33 dB_{peak} SPL was 1.54 ± 0.69 μV and its latency was 1.69 ± 0.11 msec. Cis-DDP treated guinea pigs showed stable AP responses during the early periods of treatment followed by a rather abrupt response suppression. Onset of the response suppression varied among animals but in more than half of treated guinea pigs loss of AP response occurred between day 10 and 20.

3. Cochlear potentials. The EP and cochlear microphonics recorded from the basal and third turns of the cochlea were summarized in Table I.

TABLE I

COMPARISON OF COCHLEAR POTENTIALS

Measurements	Control	cis-DDP
EP_I(mV)	84.3 ± 4.0(20)	79.8 ± 5.3(14)**
EP_{III}(mV)	68.1 ± 7.9(6)	68.8 ± 7.9(10)
CM_I (6kHz)		
sensitivity (dBSPL)	62.0 ± 3.0(20)	72.8 ± 6.0(14)**
max. output (μV)	1300 ±180 (20)	400 ±225 (14)**
CM_{III} (500Hz)		
sensitivity (dBSPL)	38.6 ± 4.3(6)	45.0 ± 5.8(10)*
max. output (μV)	820 ± 80 (6)	500 ±150 (10)**

Subscripts I and III indicate the basal and third turn of the cochlea. Sensitivity of CM is expressed by stimulus intensity necessary to elicit 100 μV peak-to-peak CM. Numbers in parenthesis are number of animals tested. * and ** indicate significant difference between two groups of animals at the probability level of 0.05 and 0.01 respectively.

4. Electrolyte concentrations in the cochlear fluids. Cis-DDP treated quinea pigs did not show significant changes in K^+, Na^+ and Cl^- concentrations in both endolymph and perilymph, as shown in Table II.

TABLE II

COMPARISION OF ELECTROLYTE CONCENTRATIONS (mM/l)

OF COCHLEAR FLUIDS

Ions	Control	cis-DDP
Potassium		
perilymph (SV)	6.1 ± 1.5(18)	5.4 ± 2.2(11)
perilymph (ST)	3.1 ± 0.6(18)	2.8 ± 0.6(12)
endolymph	157.1 ± 6.5(7)	159.2 ± 4.3(8)
Sodium		
perilymph (SV)	149.3 ±11.3(14)	156.6 ±26.1(14)
perilymph (ST)	152.2 ±13.7(14)	150.9 ± 7.4(13)
endolymph	0.3 ± 0.3(15)	0.6 ± 0.5(13)

Chloride		
perilymph (SV)	119.8 ± 5.5(5)	122.9 ± 2.7(12)
perilymph (ST)	123.2 ± 4.1(5)	121.6 ± 3.1(11)
endolymph	128.8 ± 3.3(5)	132.6 ± 4.1(9)

SV and ST indicate scala vestibuli and scala tympani respectively. Numbers in parenthesis indicate number of animals tested.

5. Morphological changes in the hair cells of the organ of Corti. In cis-DDP treated guinea pigs the loss of the outer hair cells was pronounced in the lower turns. The percentage of the missing hair cells was the highest in the first row of the first cochlear turn. Loss of the inner hair cells was observed in one guinea pig in which loss of the outer hair cells was most pronounced. There was a close correlation between loss of the outer hair cells and suppression of CM.

6. Histological changes in kidneys. The histological changes in kidneys observed in cis-DDP treated guinea pigs were minimal and did not appear to be life-threatening. In general there was slight congestion of glomerular tufts and slight dilations of renal tubules in the cortial regions.

A manuscript was submitted for publication and no further continuation of this project is planned in the near future.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the ototoxicity of cis-DDP has been reported, the pathogenesis of platinum induced deafness have not been extensively studied. The present study will make a significant contribution for the understanding of ototoxicity of cis-DDP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50073-02 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of 2.45 GHz Microwave on Brain Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Minoru Inouye	Visiting Fellow	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Enviornmental Biophysics

SECTION
Nonionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Male rats were exposed to 2.45 GHz microwave, radiation at an incident power density of 10 mW/cm^2 (SAR = 2 mW/g) daily for 3 hours from day 4 of pregnancy (in utero) through day 40 post partum. The animals were killed and the brains were removed, weighed, measured and histologically examined at 15, 20, 30 and 40 days of age. The results showed that there was no significant effect on brain development due to exposure during the embryonic, fetal and postnatal period. Japanese quail eggs were continuously exposed to 2.45 GHz microwave radiation from day 1 through day 12 of incubation (5 mW/cm^2 SAR = 4.03 mW/g). Irradiated and control embryos were removed on day 12, 13 or 14 of incubation, and the cerebella were histologically examined. In order to determine the effects of microwave exposure during embryogenesis on subsequent development of the cerebellum, some of the quail were allowed to hatch and reared to 8 weeks of age. Irradiated embryos exhibited a slight development retardation in the cerebellar cortices in terms of several morphological parameters. In the 8 week old quail, no significant differences were noted between irradiated and control cerebella in the morphological measurements of Purkinje cells.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this project was to determine the effects of 2.45 GHz microwave radiation on brain development. Pregnant Sprague-Dawley rats and fertilized Japanese quail eggs were exposed. Pregnant rats were exposed from above to a power density of 10 mW/cm^2 daily for 3 hours from day 4 of pregnancy through day 40 post partum. The animals were killed, and the brain were removed, weighed, measured and histologically examined at 15, 20, 30 and 40 days of age. The histological parameters examined included the cortical architecture of the cerebral cortex, the decline of the germinal layer along the lateral ventricle, the myelination of the corpus callosum, and the decline of the external germinal layer of the cerebellar cortex. Fertilized Japanese quail eggs were also continuously exposed to 2.45 GHz microwaves from day 1 through day 12 of incubation at 5 mW/cm^2 (SAR = 4.03 mW/g). Irradiated and control embryos were removed from eggs on day 12, 13 or 14 of incubation and the cerebella were histologically examined. Some quail were allowed to hatch and were reared to 8 weeks of age in order to determine residual changes. The extend of dendritic arbores, the length of the stem of primary dendrite and the size of the perikaryon of Purkinje cells were measured in Golgi Cox impregnated sections.

MAJOR FINDINGS AND PROPOSED COURSE: In the exposed Sprague-Dawley male progeny no remarkable differences between microwave exposed and control groups for any of the histological or quantitative parameters were observed. This data indicates that there is no significant effects on rat brain development due to 2.45 GHz microwave exposure during embryonic, fetal and postnatal periods at a incident power density of 10 mW/cm^2. In the irradiated Japanese quail embryos a slight retardation was found in the development of the cerebellar cortices in terms of several morphological parameters. The effects included the growth and subsequent decline of the external granuler layer, the differential growth of the molecular layer, the cellular differentiation and the alignment of Purkinje cells and the accumulation of granule cells beneath the Purkinje cell layer, as well as lower body and brain weights. In the 8 week old quail, no significant differences were noted between irradiated and control cerebella in the morphological measurements of Purkinje cells. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Reports in the literature has shown that developing embryos when exposed to microwave radiation have behavioral decrements when they reach maturity. This research provides information on the development of the brain which assists in the interpretation of this data as well as provide original research on the effects of microwaves on brain histology.

PUBLICATIONS

Inouye, M., Galvin, M.J. and McRee, D.I.: Effects of 2.45 GHz Microwave Radiation on the development of Japanese quail cerebellum. Teratology 25: 115-121, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50074-01 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Free Radical Metabolism of Mutagenic Acridines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Birandra K. Sinha Senior Staff Fellow LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although the mutagenic acridines intercalate into DNA it is not known whether such non-covalent interactions are the cause of frame shift mutations. The possibility that free radical metabolites of acridines are responsible for the mutagenicity of these agents has therefore been examined. Results show that free radical intermediates are formed when quinacrine and 9-aminoacridine are incubated with either the horseradish peroxidase/H_2O_2 or the prostaglandin synthetase/arachidonic acid system. Covalent binding of acridines to microsomal membranes was detected in the presence of NADPH.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Incubation of N-hydroxy-N-acetylamino-fluorene, a known carcinogen, has been shown to form free radical intermediate. In addition, other aromatic amines carcinogens form free radical intermediates. Free radicals or other oxygen derived toxic intermediates are known to induce DNA-strand breaks. Electron spin resonance spectroscopy has been used to detect the formation of free radicals from acridines.

MAJOR FINDINGS AND PROPOSED COURSE: Free radical metabolism of the acridine derivatives, quinacrine and 9-aminoacridine, has been studied using horseradish peroxidase-H_2O_2 (HRP-H_2O_2) and prostaglandin-arachidonic acid systems. In the presence of HRP-H_2O_2 quinacrine rapidly formed a free radical intermediate consisting of three lines which collapsed into a single line with a g-value of 2.0055. Under similar conditions no radical was detected with 9-aminoacridine. In contrast, incubation of either quinacrine or 9-aminoacridine with ram seminal vesicle microsomes and arachidonic acid gave a single line spectrum with g-values of 2.0055. Although no radical could be detected with rat hepatic microsomes, incubation of the acridines resulted in covalent binding to microsomal membranes which was NADPH-dependent. Free radical metabolsim and covalent binding may play a significant role in the mutagenic properties of quinacrine and 9-aminoacridine. Plans include (i) study covalent binding of acridines to DNA, RNA and proteins in the presence of peroxidase-H_2O_2 and prostaglandin synthetase-arachidonic acid systems and (ii) evaluate mutagencity of acridines in Ame's test in the presence of HRP-H_2O_2 system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since mutagenicity of acridines is not well understood, it is of great interest to understand metabolism of acridines through free radical pathways and the relavance of these intermediates to the final expression of their toxicity.

PUBLICATION

Sinha, B.K.: Free radical metabolism of mutagenic acridines and binding to microsomal membranes. Biochem. Biophys. Res. Commun. 103: 1166-1171, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50075-01 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Metabolism of Hydrazines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Ann G. Motten	NRS Postodctoral Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hydrazine derivatives which are used in industry and medicine are known carcinogens. Hydralazine, a potent antihypertensive drug is also carcinogenic. However, the mechanism of carcinogenicity is not clearly understood. The oxidative metabolism of hydralazine has been studied by means of electron spin resonance spectroscopy and spin trapping. A nitrogen-centered hydralazyl radical was detected in the presence of metal ions and red blood cells. This radical or oxygen derived species may play a role in the toxicity of this drug.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Studies of Mishra and Fridovich have suggested that free radical intermediates are formed from hydrazines in the presence of metals. More recently, carbon-centered free radical intermediates have been detected from phenylhydrazine and ethylhydrazine. The precise role of these intermediates in their toxicity is known at this time, however, they may bind to or induces changes in cellular macromolecules. Electron spin resonance spectroscopy has been employed to detect free radicals generated during the oxidative metabolism of hydralazine.

MAJOR FINDINGS AND PROPOSED COURSE: The oxidative metabolism of hydralazine, a hydrazine-containing hypotensive drug, has been studied using a spin-trapping technique. In the presence of Cu^{2+}, Fe^{2+} and Fe^{3+}, hydralazine rapidly forms a nitrogen-centered-DMPO adduct with a_N^N = 15.0G, a_H^β = 16.7G and a_β^N 2.55G. While catalase has a very small inhibitory effect, superoxide dismutase completely inhibits the formation of the DMPO adduct. Mass spectral analysis of the adduct indicates that the hydralazyl radical is trapped with DMPO. Human red blood cells also catalyze the formation of a nitrogen-centered-DMPO adduct, a_N^N = 15.9G, a_H^β = 19.4G and a_β^N = 1.7G, which is different than that obtained with metal ions. DMPO-H adduct is also formed in the red cells from hydralazine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanism of mutagenicity/carcinogenecity of hydralazine derivatives is not well understood. The free radical intermediates and consequent formation of $O_2^{\cdot-}$/$OH^{\cdot}$/H_2O_2 may play a role in their toxicity. Therefore, it is essential to study their metabolism *in vitro* and *in vivo* in order to understand their toxicity.

Future plans include (i) elucidation of the role of free radical intermediates formed from hydrazines in degradation of cellular macromolecules (ii) elucidation of the binding mechanism(s) of hydralazine to nucleic acids and proteins and (iii) identification of the enzymes involved in the metabolism of hydrazines.

PUBLICATION

Sinha, B.K. and Motten, A.G.: Oxidative metabolism of hydralazine. Evidence for nitrogen centered radical formation. Biochem. Biophys. Res. Commun. 105: 1044-1051, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50076-01 LEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Noise and Drugs on Water Control of the Cochlear Fluids.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS
	Philip E. Hamrick	Radiation Safety Officer	SFTY	NIEHS

COOPERATING UNITS (if any)

Office of Director

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to elucidate mechanisms of water movement across the endolymph-perilymph barrier in normal guinea pigs and to correlate alteration of water control of the cochlear fluids with suppressions of the cochlear responses in noise- or drug-treated guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs were anesthetized with pentobarbital sodium. In one group of guinea pigs the perilymphatic space was perfused with artificial perilymph containing tritiated water. The perfusate had the following composition (mM): NaCl 137, KCl 5, $CaCl_2$ 2, NaH_2PO_4 1, $MgCl_2$ 1, $NaHCO_3$ 12 and glucose 11. The activity of ^3H in the perfusate was 5 μCi/ml. When both scala vestibuli and scala tympani were perfused, a perfusion pipette was inserted into the scala tympani of the basal turn and a hole serving as an outlet was made in the scala vestibuli of the basal cochlear turn. The perfusion rate was 8 μl/min and the period of perfusion ranged from 3 to 20 min. Samples of the endolymph, perilymph and perfusate were collected and activities of ^3H in these samples were determined using a liquid scintillation counter. Separate animals were used for each time. An exception was made in one set of experiments in which the radioactivity in the perilymph was determined as a function of time with the same animal.

In the second group of guinea pigs samples of perilymph, endolymph, blood and cerebrospinal fluid were collected without perilymphatic perfusion. The concentrations of Na^+, K^+ and Cl^- were measured in these samples. The osmolarity of endolymph and perilymph was determined by measurement of the freezing point depression, whereas the osmolarity of blood serum and cerebrospinal fluid was estimated by the vapor pressure measurement.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Diffusional permeability to water of the endolymph-perilymph barrier. When the perfusate was introduced into the scala tympani of the basal turn, the concentration of ^3H in fluid samples taken from the scala tympani increased rapidly and reached 95% relative to the concentration of ^3H in the perfusate within 3 min. The concentration of ^3H in fluid samples from the scala vestibuli had a tendency to increase exponentially as the period of perfusion increased, the time constant being 0.566 min^{-1}. The concentration of ^3H in the endolymph increased rapidly and reached 80% during the first 5 min perfusion and thereafter the rate of increase was slower. The mean normalized concentration of ^3H was 93% in the endolymph 15 min after perfusion commenced. The mean value of the rate constant was 1.367 min^{-1} which is greater than the rate constant for K^+.

2. Osmolarity of the cochlear fluids. The preliminary results show that the osmolarity of the endolymph was 300.2 ± 6.9 mOsM which was slightly higher than the osmolarity of the perilymph (291.6 ± 6.1 mOsM). The osmolarities of blood serum and cerebrospinal fluid were 281.8 ± 11.8 mOsM and 294.5 ± 8.2 mOsM respectively.

We plan 1) to estimate the diffusional permeability coefficient for water of the endolymph-perilymph barrier, 2) to determine if the movement of tracer is altered by inhibition of active ion transport (by anoxia or ouabain) and 3) to study possible alterations of water control in the cochlear fluids in noise- or drug-treated animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanisms involved in the kinetics of water in the cochlear fluids have been a subject of extensive studies but convincing experimental data are still limited. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ear under exposure to physical and chemical agents.

TITLE: "Photodegraduation of Adsorbed Polycyclic Arenes"

PRINCIPAL INVESTIGATOR: Joan M. Daisey, Ph.D., Institute of Environmental Medicine, NYU Medical Center

PROJECT OFFICER (NIEHS): Colin F. Chignell, Ph.D., Chief, Laboratory of Environmental Biophysics

DATE GRANT INITIATED: December, 1978 (Renewed December, 1979)

CURRENT ANNUAL LEVEL: $26,000

GRANT DESCRIPTION

OBJECTIVES: Polycyclic aromatic hydrocarbons (PAH) in the atmosphere can be associated with particle substrates which differ considerably depending upon source. These differences in substrate composition can have a substantial impact upon the PAH half-lives, reaction products and biological activity. As there is little or no information on the half-lives of adsorbed PAH, an investigation has been initiated of the photodegradation of these compounds under simulated environment conditions. The objectives of this study are:

1. To design, construct and evaluate a fluidized-bed photochemical reactor for laboratory studies of photodegradation of adsorbed PAH;
2. To determine the stability of some PAH epoxides (possible intermediates in photodegradation) adsorbed onto various substrates;
3. To investigate the rates and products of photodegradation of adsorbed PAH on several substrates under various conditions of temperature and humidity.

METHODS EMPLOYED: The photreactor consists of a glass column 24 cm in diameter. Particles are suspended to a height of about 10 cm by the flow of air or other gases through a fritted disc at the base of the column. An alpha particle emitting source in the reactor eliminates static charging of the particles. The column of moving particles is irradiated by a 200 watt quartz mercury vapor lamp with a reflector which is mounted 15 cm from the center of the column. The glass reactor acts as a filter for ultraviolet wavelengths giving a spectrum in the reactor which is similar to sunlight. Light intensity in the reactor from 297 to 366 nm is about 30 watts/m^2, approximately half the intensity of solar irradiance for this region of the spectrum at 0° zenith angle.

MAJOR FINDINGS AND PROPOSED COURSE: Using the photoreactor, the rates of degradation of pyrene on glass beads and on Carbosieve S have been determined. In general the rates are reproducible to within ± 20-30% from one experiment to the next. When the reaction is followed over the same time interval, rates appear to be reproducible to within 20%.

The rate of degradation was found to follow a first order kinetic rate law but the half-life of pyrene was strongly dependent upon the substrate. The half-life of pyrene in the presence of air and light was 1.0 ± 0.2 hrs. on glass beads and 31 ± 6 hrs. on Carbosieve S, a pure carbon substrate.

Within the experimental uncertainties of the determinations, the rate of degradation of pyrene in the presence of light did not differ from that in the absence of light. In the case of the glass beads, in an experiment in which nitrogen was substituted for air, there was no detectable loss of pyrene over a period of several hours. Thus, oxygen appears to be required for the degradation reaction although light is not.

Extracts of samples of pyrene on glass beads and on Carbosieve S which had been exposed to air and light in the photoreactor were analyzed by HPLC. In the case of the glass substrate the chromatograms indicated the formation of 7 products. Although standard pyrene derivatives were not available at this time, the chromatograms suggest that quinones and bipyrene may have been formed.

For the Carbosieve S, there was no evidence of the presence of quinones in the extracts. Several pyrene quinone derivatives as well as 1,1-bipyrene were available at the time of these experiments. It is not clear at present whether these compounds are not recovered from the carbon substrate or do not form. The formation of bipyrene was observed both with and without light. Compounds eluting at the same retention times as fluoranthene and benzo(e)pyrene were also observed, suggesting the possibility of rearrangement reactions of pyrene on the surface of carbon. Experiments to confirm the identities of these compounds are in progress.

These first experiments on the degradation of pyrene under simulated environmental conditions have shown that the photoreactor which has been developed can provide potentially useful information on the rates and products of degradation of PAH and the factors which influence them. Further experiments with other PAH's, under a variety of conditions, can provide needed information for the assessment of environmental and occupational health hazards of airborne PAH in the context of changing fuel use patterns. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Effluents from such processes as oil shale conversion and coal gasification contain high concentrations of PAH. Once released into the environment these agents may be adsorbed onto soil particles and other substrates. Since photochemical degradation in the presence of oxygen could give rise to intermediates that are more chemically reactive than the parent compounds it is of importance to isolate and identify such compounds.

LABORATORY OF ENVIRONMENTAL CHEMISTRY

LABORATORY OF ENVIRONMENTAL CHEMISTRY
Summary Statement

The general goal of the laboratory is to use inter- and intra disciplinary strategies in chemistry and biology to understand the causes of environmentally related human health diseases at the molecular level so that preventive or control measures may eventually evolve. Operationally the laboratory has two major interactive research programs viz. chemical disposition and mechanisms and specialty analytical instrumentation. The chemical disposition and mechanisms program combines the efforts of existing programs in biochemistry, bioorganic chemistry and synthesis and in time will establish a new activity in physical and theoretical chemistry. Biochemistry efforts include pharmacokinetic considerations, investigation of the role of metabolism and enzyme mediated events in the mechanism of toxic action of environmental chemicals and quantitative aspects of both covalent and non-covalent chemical binding to biopolymers. Parallel organic efforts provide detail stereochemical elucidation of mechanisms including structural characterization of intermediary and stable terminal products and conjugates; investigate qualitative aspects of binding and synthesize needed compounds. Mechanistic findings are related to structure and toxicity through study of molecular interactions and properties using physical and biophysical measurements and theoretical chemistry approaches including selected measurements with mass spectrometry (MS), high field nuclear magnetic resonance (NMR) etc. and derivation or measurement of relevant electronic properties such as electrostatic potential contour maps.

The special analytical instrumentation program will conduct in depth and relevant research primarily in mass spectrometry and nuclear magnetic resonance spectroscopy. This includes both basic and applied research toward development of new and improved methods in analytical MS and NMR and their application to biological problems as well as relevant new and improved methods in instrumentation design and operation. More emphasis will be placed on establishing "chemical appearances" of environmental/biologically interesting compounds through selected MS and NMR measurements as a complement to the mechanism program.

In addition to the research commitment of these programs, the laboratory is also commited to providing analytical and consultative support to other Institute programs. Support capabilities cover the range of research activities and expertise, but mass spectrometry, nuclear magnetic resonance spectroscopy and analytical biochemistry are emphasized. The work group leaders have ultimate responsibility for maintaining a responsive support program and make specific support function assignments in consultation with the Laboratory Chief.

RESEARCH ACTIVITIES

Analytical Chemistry

The analytical chemistry program continues to develop new and improved analytical methods to facilitate all aspects of environmental/biological research. Advances in biomedical sciences reportedly have followed the development of analytical methods. State of the art instrumentation such as the high resolution and tandem quadrapole mass spectrometer are used to achieve high

sensitivity and specificity in quantitative analysis. Negative chemical ionization mass spectrometry has evolved to the point of being available as a tool in support of other Institute research programs. By coupling this system to a high pressure liquid chromatography/(HPLC), it is now possible to study a wide range of new problem areas with this ionization technique. The capability in trace organic analysis can frequently permit identification of an unknown compound on the basis of its mass spectrum. This program is also doing fundamental research in gas phase ion chemistry as a means of improving predictive abilities in analytical applications of mass spectrometry and to study the intrinsic chemistry applications of ionic species.

Specific accomplishments in mass spectrometry include upgrading a double-focusing mass spectrometer by the addition of a fast atom bombardment ion source and obtaining the mass spectra of various polar compounds, interfacing a positive/negative chemical ionization mass spectrometer to a liquid chromatograph via a HPLC/MS variable split-type interface and analyzing for various explosives and pesticides. Collisional induced dissociation of certain explosives was also done using mass analyzed ion kinetic energy spectrometry. This technique was also used to study the decomposition reaction of 2,3,7,8-tetrachlrodibenzo-p dioxin as a confirmating method. Methane negative chemical ionization mass spectrometry was used to investigate the mass spectrometry of organophorus pesticides and the formation of the $PO_3(-)$ anion.

Two often asked questions about impact of substances foreign to the biological system concerns their metabolites and how they interact with the body. Nuclear magnetic resonance spectoscopy probably the single most useful technique available to the chemist for determining the structure of organic compounds and for studying interaction between biological materials and foreign substances is also being applied and new techniqes developed to solve specific problems. ^{13}C chemical shifts were reported for several potential or demonstrated oxidative metabolites of acenaphylene, phenanthrene and pyrene. Included were the arene oxide, diol diacetates, quiones, phenols, and mercapturic acid derivatives. Comparison of the data for similarly substituted phenanthrenes and pyrenes suggest that the substituent effects on ^{13}C chemical shifts for the substituents examined are relatively constant from one ring system to another. Differences in substituent effects were observed between substituted five- and six-membered rings. The data were used to derive substituent parameters which should prove useful in structural studies of metabolites derived from other polycyclic aromatic hydrocarbons.

Installation of a high field superconducting NMR spectrometer operating at 360 MHz has been completed which will increase our capabilities to solve biological problems by providing greater chemical shift dispersion, greater sensitivity for various nuclei and the ability to use large sample tubes.

Developments in analytical biochemistry include a method for reproducible and quantitative determination of polybrominated biphenyls in animal tissue, identification of an internal standard for the determination by gas chromatography(GC)-mass spectrometry of TCDD at part-per-trillion levels, and the HPLC and GC separations and determination of urinary metabolites of phthalate esters. The general utility of multi-isotopic labeling to eliminate interferences has been studied using $^{12}C/^{13}C/^{14}C$- diethylhexyl phthalate.

Biochemistry

The laboratory's biochemistry program serves as a common communication point between analytical and organic chemists and the Institute's biologically oriented laboratories. It develops methodologies to respond to the trace level analysis needs in biological systems, and it performs research on the method of action of environmental toxins at the molecular level with emphasis on metabolic factors. Of particular interest has been the metabolic aspects of the possible genotoxic potential of diethylhexyl phthalate (DEHP), an important industrial chemical employed with entensive utility, primarily as plasticizer. Incorporation of the labled carbon atom from di-(2-ethyl-[1-^{14}C]-hexyl) phthalate into DNA in rat liver appears to be a real phenomenon. It has proven impossible thus far to cause this to occur in vitro. Preliminary studies indicate that the major if not sole products of the incorporation are the normal nucleic acid bases. Evidence for a chemical genotoxicity is lacking. Future studies will involve a "regenerating liver" system in order to produce more highly labled DNA for degradation studies. Studies on the association of DEHP metabolites with DNA will be extended to mice. DEHP metabolism in a variety of species will continue to be studied in order to select a model more appropriate than the rat. In vitro studies to more clearly elucidate the metablic pathways are in progress.

Other research has focused on the description of mechanisms at various biochemical and molecular levels including development of structure-activity correlations as a predictive tool in toxicology. Emphasis is placed on identifying relevant in vitro binding which correlates with in vivo potency. The use of physcial/theoretical chemistry approaches to derive relevant reaction potential properties for binding molecules will be explored. There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. The guinea pig was used as an extremely sensitive animal model to investigate the toxic effects of polybrominated biphenyl (PBB) mixtures and individual isomers and congeners. Lethality of the mixture in guinea pigs appears to be associated with toxic coplanar molecular conformers of certain favorably substituted biphenyl components. The suspected isomers are being synthesized for individual testing in the guinea pig to complete work. Toxicity of this type is similar if not identical, to that observed for related planar halogenated aromatic hydrocarbon demonstrated to bind the dioxin receptor.

A human plasma protein appears to bind specifically to toxic planar/coplanar halogenated hydrocarbons of the dioxin type. The exact nature of this binding is being studied using x-ray crystallographic techniques and theoretical chemistry approaches. Other work will attempt to show the relationship of such binding to the mechanism of dioxin toxicity.

Other work concerned with the development of immunoassay method for compounds of environmental interest and delineation of mouse glulathione S-tranferase activities has been terminated.

Organic Chemistry

The laboratory's organic chemistry program develops new and improved methods in

synthetic chemistry to support diverse studies on biomechnism elucidation at the molecular level with particular attention to stereochemistry. Such work includes synthetic methods and supporting organic analysis work particularly with high pressure liquid chromatorgraphy for reactive intermediary metabolites and polar metabolties and their conjugates. The program is especially interested in identifying reactive electrophilic species from metabolic activation that may mediate toxic effects. Of particular interest has been the metabolism of polycyclic aromatic hydrocarbons and detoxification by the glutathione S-transferase enzymes. The theory that the metabolism of chemicals in the body can lead to the formation of more toxic substances through metabolic activation or less toxic substances through detoxification has been widely accepted. The mechanistic aspects of the GSH transferase reaction underscore the relevance of sterochemical factors in the influencing the rate of elimination of enantiomeric oxides via the glutathione pathway. The absolute stereochemsitry of the glutathione adducts of (±) benzo[a]pyrene-4,5-oxide was established by using enantiomerically pure oxide(s) as substate for the alkylation reaction. In general, the gluathione transferases show a preference for the oxirane carbon with the R configuration. This stereopreference is not restricted to (±) benzo[a]pyrene-4,5-oxide but has also been detected with (±)-benz[a]anthracene 5,6-oxide and the pro-chiral substrates pyrene-4,5-oxide abd phenanthrene-9,10-oxide. It is of more than casual interest to observe that this stereopreference is complementary to that of the epoxide hydrolase, another key detoxifying system, which shows stereoselectivity for the S configuration oxirane carbon.

Polynuclear aromatic hydrocarbons are recognized to be of major environmental importance due to their widespread ecological distribution and, in some cases, their concentration in the food chain. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit critical biological and toxicological studies. Synthetic availability of appropriate model compounds allows further examination of the mechanisms of their biological activity. The new methodology developed for the synthesis of benzoanthracenes has been broadened and extended to prepare more complex examples of polynuclear aromatic hydrocarbons. These have included highly alkylated benzanthracenes and benzanthracene derivatives capable of exhibiting optical activity. Functionalized benzanthracenes with the potential for conversion into other polynuclear aromatic hydrocarbons have been synthesized. Known and hypothetical oxygenated metabolites of some of these hydrocarbons have been prepared. Finally heteroaromatic polynuclear hydrocarbons have been synthesized.

An active program in bioorganic chemistry contributes to maintain a high level of expertise in critically important areas such as biomechanisms, synthetic organic chemistry, and chromatographic techniques. The benefits of this approach are reflected in consulting and collaborative activities with research groups throughout the Institute. Further work on the mechanism(s) involved in the separation of small peptides under reversed-phase HPLC conditions has resulted in the development of improved conditions for the analysis of the glutathione adducts of epoxides and small polypeptides of bombesin series. The polypeptide bombesin may serve as a marker for small cell lung cancer. For the glutathione conjugates of (±)-styrene oxide and (±) benzo[a]pyrene-4,5-oxide the four diastereomers derived from each epoxide are reproducibly separated. For (±) benz[a]anthracene-5,6-oxide the corresponding four diastereomers are separated using two different solvent conditions on the same column. For the bombesin-like polypeptides a pH induced shift has been used to identify the presence of

a histidine residue. An increase in the retention time of the sample is observed as a result of neutralization of the histidine residue. Additionally, chemoselective methods for the oxidation of bombesin were used in an effort to identify potential artifacts formed during isolation. The proposed course of research involves a correlation between the order of elution under reserved-phase conditions and the absolute configuration of glutathione adducts at the carbon-bearing sulfur.

OTHER ACTIVITIES

Contracts are sometimes required to interact with the various research, collaborative/support components to maintain a viable program. Contractors are nearing completion of the analysis of large numbers of human milk, blood (serum) and placenta tissue samples for polychlorinated biphenyls, DDE and total organic chlorine content using newly developed and validated methods. These methods have been documented and submitted for publication. Other contract work has thus far been unsuccessful in its attempts to develop a solid-state RIA for dioxins or to obtain a monoclonal antibody to the 2,3,7,8-tetrachloro isomers. Problems associated with application of the RIA to soil samples have delayed the analytical work, but these problems are thought to have been largely overcome and analysis of soil samples is proceeding. If time permits, the RIA for dioxins will be applied to a series of samples of human milk.

The chemical and biological properties of molecules are governed by their electronic structures. In order to understand how molecules interact biologically or the mechanism by which molecule are activated metabolically and subsequently interact biologically, it is necessary to be able to calculate reliably the electronic structure of the molecules themselves. Collaborative studies with theoretical chemists have been initiated to calculate such structures for certain halogenated aromatic hydrocarbons with which we have worked for numerous years and have collected considerable chemical and biological data. We hope that such work will be the beginning of a continuing effort in predictive toxicology.

A substantial part of the overall efforts of these work groups involves collaborative/support activities. Collaborative projects can be initiated by individual scientists or may have Institute priority in terms of a "targeted research area" having immediate interest and seeking immediate data to bear on an imminent health problem. The routine support requests generally fall into one of two categories: short-term requests such as compound purity determination and organic synthesis by published procedures, and a variety of long-term requests which often require new and improved methodology development such as qualitative and quantitative analysis of complex mixtures and organic synthesis by unpublished procedures. In the latter case, it is difficult to determine the amount of time and effort which will be necessary to complete the task. A trial period may be necessary to demonstrate feasibility within the capabilities of the group. Therefore, for obvious reasons, these latter requests are carefully evaluated, and we frequently seek to do this work on a collaborative research support basis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10003-03 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Synthetic and Analytical Studies in Bioorganic Chemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	O. Hernandez	Visiting Scientist	LEC NIEHS
OTHER:	A. Bhatia	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to explore and develop synthetic and analytical methods for the study of biological processes. The nature of this methodology is determined by specific requirements from current projects within the Laboratory. In addition, expertise in bioorganic chemistry is reflected in consulting and collaborative activities with other research groups at the Institute.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: High pressure liquid chromatography (HPLC), carbon magnetic resonance, proton magnetic resonance, and the usual synthetic apparatus and equipment.

MAJOR FINDINGS AND PROPOSED COURSE: Further work on the mechanism(s) involved in the separation of small peptides under reversed-phase conditions has resulted in the development of improved conditions for the analysis of the glutathione adducts of epoxides and small polypeptides of the bombesin series.

For the glutathione conjugates of (±)-styrene oxide and (±)-benzo[a]pyrene 4,5-oxide the four diastereomers derived from each epoxide are reproducibly separated. For (±)-benz[a]anthracene 5,6-oxide the corresponding four diastereomers are separated using two different solvent conditions on the same column.

For the bombesin-like polypeptides a pH induced shift has been used to identify the presence of a histidine residue. An increase in the retention time of the sample is observed as a result of neutralization of the histidine residue. Additionally, chemoselective methods for the oxidation of bombesin were used in an effort to identify potential artifacts formed during isolation. The proposed course of research involves a correlation between the order of elution under reversed-phase conditions and the absolute configuration of glutathione adducts at the carbon-bearing sulfur.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An active program in bioorganic chemistry contributes to maintain a high level of expertise in critically important areas such as biomechanisms, synthetic organic chemistry, and chromatographic techniques. The benefits of this approach are reflected in consulting and collaborative activities with research groups throughout the Institute.

PUBLICATIONS

Hernandez, O., Yagen, B., Cox, R.H., Bend, J.R., and McKinney, J.D.: HPLC analysis of the isomeric thioether metabolites of styrene oxide. J. Liquid Chromatogr. 5, 345-365 (1982).

Kohli, K.K., Hernandez, O., and McKinney, J.D.: Fractionation by HPLC of microsomal cytochrome P-450 induced by hexachlorobiphenyl isomers. J. Liquid Chromatogr. 5, 367-377 (1982).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10004-03 LEC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies in Nuclear Magnetic Resonance (NMR) Spectroscopy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. McKinney	Research Chemist	LEC NIEHS
Other:	O. Hernandez	Visting Scientist	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{13}C-Chemical shifts were reported for several potential or demonstrated oxidative metabolites of several polycyclic aromatic hydrocarbons. Installation of a high field NMR spectrometer has been completed.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{1}H and ^{13}C high-resolution nuclear magnetic resonance (NMR) spectroscopy. High-Field, super conducting NMR spectrometer.

MAJOR FINDINGS AND PROPOSED COURSE: ^{13}C chemical shifts are reported for several potential or demonstrated oxidative metabolites of acenaphthylene, phenanthrene and pyrene. Included are the arene oxide, diol diacetates, quinones, phenols, and mercapturic acid derivatives. Comparison of the data for similarly substituted phenanthrenes and pyrenes suggest that the substituent effects on ^{13}C chemical shifts for the substituents examined are relatively constant from one ring system to another. Differences in substituent effects were observed between substituted five- and six-membered rings. The data were used to derive substituent parameters which should prove useful in structural studies of metabolites derived from other polycyclic aromatic hydrocarbons.

Installation of a high-field superconducting NMR spectrometer operating at 360 MHz has been completed and routine spectra are being generated. A search is underway for a permanent scientist in NMR spectroscopy to lead a major research program in NMR at the Institute.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NMR spectroscopy is probably the singly most useful technique available to the chemist for determining the structure of organic compounds and for studying molecular interactions. The data obtained during this reporting period should prove useful in our studies in the synthesis of metabolic intermediates of ervironmental significance. High field NMR capabilities at the Institute will increase our capabilities to solve biological problems by providing greater chemical shift dispersion, greater sensitivity for various nuclei and the ability to use large sample tubes.

PUBLICATIONS

Cox, R.H. and Hernandez, O. ^{13}C NMR studies of potential metabolites of polycyclic aromatic hydrocarbons; acenaphthylene, phenanthrene, pyrene and benzo[a]pyrene. J. Org. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10007-02 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

High Pressure Liquid Chromatography/Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.E. Parker	Research Chemist	LEC NIEHS
OTHER:	J.R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)
Research Services Branch

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A positive/negative chemical ionization mass spectrometer has been coupled to a liquid chromatograph via a HPLC/MS variable split-type interface. The system is being further refined and tested for its use in analysis of thermally labile compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: This project involved restoration of the Finnigan 3300 chemical ionization mass spectrometer to allow routine analysis of positive and negative ions, and the design and construction of an inlet system to accomodate a Hewlett-Packard HPLC/MS variable split-type interface.

MAJOR FINDINGS: The system is now working in both positive and negative ion chemical ionization modes, and LC/MS has been used for the analysis of explosives and pesticides. A similar interface has been designed and used for LC/MS/MS.

On line HPLC/NCI-MS has been used for the analysis of explosives mixtures. HPLC mobile phases were acetronitrile:water (50:50) and methanol:water (50:50), which served also as NCI reagent gases. Standard mixtures containing TNT, RDX, tetryl and PETN and a military explosive, tetrytol, have been analyzed by the LC-MS system. The minimum detectable amount of TN - was 100 ng injected on column, or approximately 1 ng to the ion source.

Eighteen organophosphorus pesticides were studied by combined HPLC/NCI-MS. LC separation was done on a reversed-phase C-8 column, using acetonitrile: water (60:40) as the mobile phase. The negative ion mass spectra obtained under these LC/MS conditions are very simple and are very similar to those reported for methane-enhanced negative ionization. Molecular ions are generally not present in either mode, but intense fragment ions containing useful structural information are usually observed.

Five classes of triazine herbicides were studied by combined LC/MS. Separations were done on a reversed-phase C-8 column, using acetonitrile:water as the mobile phase, followed by in-line UV and mass spectral analysis. Positive and negative ion mass spectra were recorded. For most of the triazines both positive and negative ion spectra obtained with the direct liquid introduction LC/MS probe interface gave molecular ion information. Most of the triazine studies were more sensitive in the positive ion mode than in the negative ion mode.

An on-line liquid chromatography/tandem quadrupole (LC/MS/MS) instrument was used to monitor aromatic acids in a complex mixture. This mixture contains components known to exist as humic acid degradation products. The use of the tandem quadrupole removed LC solvent interferences and selected the protonated molecular ion of a particular acid to undergo collisional activation (CA). The resulting fragments provide the specificity to detect target acids; the CA spectrum is "clean" and resembles the reference spectrum. This specificity is unobtainable by LC/MS alone. Present LC/MS/MS detection limits are 5 μg injected on column (50 ng in source) for a full scan detecting fragment ions greater than 5% relative intensity.

PROPOSED COURSE: Work is continuing to determine the effect of source geometry, temperature, pressure and LC solvent systems on sensitivity and specificity. Work is proceeding on the development of a micro LC system which should increase the overall sensitivity of the LC/MS system.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The LC/MS instrument should allow on-line mass spectrometric analysis of thermally labile compounds which cannot survive GC/MS.

PUBLICATIONS

LC/MS

Parker, C.E., Haney, C.A., and Hass, J.R.: High Pressure Liquid Chromatography/ Negative Chemical Ionization Mass Spectrometry of Organophosphorus Pesticides, J. Chromatog. 237(2), 233-248 (1982).

Parker, C.E., Haney, C.A., Harvan, D.J. and Hass, J.R.: High Pressure Liquid Chromatography/Mass Spectrometry of Triazine Herbicides. J. Chromatog. 242(1), 77-96 (1982).

LC/MS/MS

Voyksner, R., Hass, J.R., and Bursey, M.M.: An On-Line Liquid Chromatography/ Mass Spectrometry/Mass Spectrometry Experiment. Analytical Letters, 15(A1), 1-12 (1982).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10009-02 LEC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mouse Glutathione S-Transferases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.D. McKinney Supervisory Research Chemist LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No additional work was completed this reporting period. Project has been terminated due to lack of resources.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Conventional procedures were employed for enzyme purification. Steady-state kinetics was studied spectrophotometrically. A standard procedure for immunization was used for raising antisera in rabbits against each form of glutathione S-transferase.

MAJOR FINDINGS AND PROPOSED COURSE: Our past work indicated large species variation for this enzyme system in rats and mice. This project has been terminated due to lack of resources.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Glutathione S-transferase is known to be an important enzyme system for the detoxification of numerous xenobiotics. Large species variations of this enzyme system (substrate specificity and multiple forms) may become an important concern in choosing a proper animal species for evaluations of biochemical transformation and toxicity of xenobiotics.

PUBLICATIONS

Lee, C.-Y., Johnson, L., Cox, R.H., McKinney, J.D. and Lee, S.-M.: Mouse liver glutathione S-transferases. J. Biol. Chem. 265(15), 8110-8116, (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10011-01 LEC

PERIOD COVERED
October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Methods for Determination of Polybrominated Biphenyls in Animal Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.D. McKinney Supervisory Research Chemist LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Chemistry

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INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Liver and renal adipose tissues taken from male Fischer rats given Firemaster FF-1 a polybrominated biphenyl (PBB) each workday for 180 days by gavage and by diet were analysed by electron capture gas chromatography for the major component 2,4,5,2',4',5' hexabromobiphenyl (HXBB). For validation purposes the results of two extraction methods, viz: (a) hexane, for both liver and adipose extraction and (b) chloroform: methanol (C:M) for liver and methylene chloride (CH_2Cl_2) for adipose extraction were compared. As an independent monitor of these results, total tissue bromine was determined by neutron activation analysis (NAA). Extraction efficiencies were compared with each other and with the NAA values. C:M-CH_2Cl_2 extractions were both reproducible and quantitative for both PBBs and tissue lipids and agreed well with NAA results. Hexane extractions gave lower lipid and HxBB values and had a high coefficient of variance (CV) indicating poorer extractability and reproducibility.

PROJECT DESCRIPTION

METHODS EMPLOYED: Liver and adipose tissues from rats dosed with Firemaster FF-1 by gavage (10 and 1 MG/KG/Day) and in rat chowfeed (1 MG/KG Feed) were analysed for the major component, 2,4,5,2',4',5' hexabromobiphenyl (HxBB) by electron capture gas chromatography. Results were compared for validation purposes between two extraction methods, viz: (a) Hexane for both liver and adipose, a method which was found to yield interlaboratory standard deviations of 15% or better for chlorinated pesticides and (b) Chloroform:Methanol (C:M) for liver and methylene chloride (CH_2Cl_2) for adipose which were shown to give quantitative lipid recoveries. A steady state was assumed to exist for the PBBs in the rat study since it was shown to occur in milk fat in cows by daily feeding of PBBs. Neutron activation analysis for this reason was chosen as an independent method to monitor the efficiency of the extraction methods to each other and to total tissue bromine.

MAJOR FINDINGS AND PROPOSED COURSE: Hexane extracted tissues gave lower lipid and HxBB recoveries than those of the C:M-CH_2Cl_2 extraction. The coefficient of variance (CV) values were about three times higher indicating poorer reproducibility for the hexane extraction method. When compared with total bromine, HxBB (expressed as equivalent bromine) results were reproducible and quantitative for adipose levels when extracted with C:M-CH_2Cl_2. Similarly liver to adipose ratios of HxBB expressed on the lipid weight basis gave comparable means and CV's for the various treatment groups. Hexane extraction of equivalent bromine of HxBB showed wide CV's when compared with total bromine and for liver to adipose ratios expressed on the lipid weight basis. These results show that for the selection of efficient extraction solvent systems the compound(s) to be analysed and the tissue to be extracted must be considered depending upon the lipophilic and/or hydrophilic nature of both compound and tissue. In addition the extraction procedure should be monitored by some additional means, for example by use of a radioisotope.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The need for reproducible and quantitative tissue extraction procedures is obvious when assigning toxic and/or relatively toxic levels of a given compound with other related compounds or metabolites. Dependable analytic procedures are essential for any medico-legal problems which might arise from the use of these compounds.

PUBLICATIONS

Fawkes, J., Albro, P.W., Walters, D.B. and McKinney, J.D.: Comparison of Extraction Methods for Determination of Polybrominated Biphenyl Residues in Animal Tissue. Analytical Chemistry, accepted, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES-30003-11 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Development of Analytical Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.W. Albro	Research Chemist	LEC NIEHS
OTHER:	J.R. Hass	Research Chemist	LEC NIEHS
	K. Chae	Chemist	LEC NIEHS
	Y. Tondeur	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This general project area has as its objective, to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Recent work has emphasized development and validation of methods for (a) quantitative and reproducible extraction and clean-up of halogenated aromatic compounds from soil and adipose tissue for subsequent GC-MS or immunoassay, and (b) fortifying samples under conditions yielding equilibration of "spike" with endogenous compound. In addition, applications of capillary column gas chromatography and two-dimensional gas chromatography have been investigated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: Tetrachloromonofluorodibenzo-p-dioxin has been evaluated as an internal standard for the dtermination by gas chromatography-mass spectrometry of TCDD at part-per-trillion levels and found suitable under appropriate conditions. HPLC and GLC methods for the separation and determination of urinary metabolites of phthalate esters have been developed and characterized. The general utility of multi-isotopic labeling to eliminate interferences has been studied using $^{12}C/^{13}C/^{14}C$ - diethylhexyl phthalate. In general, we intend to eliminate this project as a separate program area and treat it as a functional part of the specific research projects to which it contributes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Laboratory of Chemistry as well as be of utility for other studies within the Institute.

PUBLICATIONS

Chae, K., and Albro, P.W.: Synthesis of fluorotetrachlorodibenzo-p-dioxin. J. Environ. Sci. Health. (in press).

McKinney, J.D., Albro, P.W., Cox, R.H., Hass, J.R., and Walters, D.B.: Problems and pitfalls in analytical studies in toxicology. in The Pesticide Chemist and Modern Toxicology, S.K. Bandal, G.J. Marco, L. Golberg and M.L. Lang (eds.), ACS Symp. Series 160, Am. Chem. Soc., Washington, D.C. (1981) pp. 439-460.

Albro, P.W., Jordan, S.T., Schroeder, J.L., and Corbett, J.T.: Chromatographic separation and quantitative determination of the metabolites of di-(2-ethylhexyl) phthalate from urine of laboratory animals. J. Chromatogr. (in press).

Albro, P.W., Hass, J.R., Peck, C.C., Jordan, S.T., Corbett, J.T., and Schroeder, J.: Applications of isotope differentiation for metabolic studies with di-(2-ethylhexyl) phthalate. (Submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30015-08 LEC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies in Chemical Ionization Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Research Chemist	LEC NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

M.M. Bursey, Department of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study of the negative ion chemistry of compounds producing PO_3^- is ongoing. Other work is limited because of reassignment of resources to the HPLC/MS project.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromotography/mass spectrometry techniques negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Investigations of the mass spectrometry of organophorus pesticides by MNCI is ongoing. The reactions leading to the formation of the PO_3-anion and fragmentation of various organophorus compounds are being studied.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Negative ion chemical ionization mass spectrometry was shown to permit analysis of several classes of environmental contaminants at lower levels than previously possible. For others, a better understanding of the interpretation of the mass spectra at the ppb level. Chemical ionization techniques have provide information complementary to that obtained using electron impact ionization methods. The methods employed in this project allows one to gather further information concerning the nature of an unknown sample.

PUBLICATIONS

Busch, K.L., Parker, C.E., Harvan, D.J., Bursey, M.M., and Hass, J.R.: "Negative ion mass spectra of organic nitriles." Applied Spectroscopy, 35, 85-88, (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30020-11-LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Transport and Metabolism of Phthalate Esters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.W. Albro	Research Chemist	LEC NIEHS
OTHER:	J.R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

C.C. Peck, M.D., LTC, MC, USUHS

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Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Distributions of metabolites of DEHP in urine have been compared for rats, mice, hamsters, guinea pigs and monkeys. All except rats form and excrete mainly glucuronide conjugates. Guinea pigs perform very little oxidative metabolism prior to excretion. Studies on the incorporation of label from ^{14}C-DEHP into DNA in rat liver in vivo suggest $^{14}CO_2$ as a precursor, but are incomplete.

PROJECT DESCRIPTION

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques, HPLC.

MAJOR FINDINGS AND PROPOSED COURSE: Incorporation of the labeled carbon atom from di-(2-ethyl-[1-^{14}C]-hexyl) phthalate into DNA in rat liver appears to be a real phenomenon. It has proven impossible thus far to cause this to occur in vitro. Preliminary studies indicate that the major if not sole products of the incorporation are the normal nucleic acid bases. Evidence for a chemical genotoxicity is lacking. Future studies will involve a "regenerating liver" system in order to produce more highly labeled DNA for degradation studies. Studies on the association of DEHP metabolites with DNA will be extended to mice. DEHP metabolism in a variety of species will continue to be studied in order to select a model more appropriate than the rat. In vitro studies to more clearly elucidate the metabolic pathways are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PUBLICATIONS

Albro, P.W., Hass, J.R., Peck, C.C., Odom, D.G., Corbett, J.R., Bailey, F.J., Blatt, H.E., and Barrett, B.B.: Identification of the metabolites of di-(2-ethylhexyl) phthalate in urine from the African Green Monkey. Drug Metab. Disp. 9:223 (1981).

Peck, C.C., Odom, D., Albro, P.W., Jess, D.A., and Barrett, B.B.: Effect of heat on the conversion of di-2-ethylhexyl phthalate to mono-2-ethylhexyl phthalate in human plasma. Transfusion: 21:163 (1981).

Albro, P.W., Corbett, J.T., Schroeder, J.L., Jordan, S., and Matthews, H.B.: Pharmacokinetics, interactions with macromolecules, and species differences in metabolism of DEHP. Environ. Health Persp. (in press).

Peck, C.C., and Albro, P.W.: The toxic potential of the plasticizer di-2-ethylhexyl phthalate in the context of its disposition and metabolism in primates and man. Environ. Health Persp. (in press).

Albro, P.W., Corbett, J.T., Schroeder, J.L. and Jordan, S.T.: Incorporation of radioactivity from labeled di-(2-ethylhexyl) phthalate (DEHP) into DNA of rat liver in vivo. (submitted).

Sherman, L., Thompson, K., O'Kell, R.T., Albro, P., and Inkster, M.: Phthalate levels in microwave-thawed fresh frozen plasma. Transfusion (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 30034-07 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemistry of Aromatic Compounds and Their Environmental Transformation Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L.A. Levy	Research Chemist	LEC NIEHS
OTHER:	S. Kumar	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development of rational synthetic routes to polynuclear aromatic hydrocarbons and their metabolites have been investigated. Models appropriate to the study of the chemical and physical properties of these classes of compounds as potential persistent environmental agents have been prepared.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, nuclear magnetic resonance spectroscopy, other spectroscopy methods (IR, UV), chromatography (column, glc, hplc).

MAJOR FINDINGS AND PROPOSED COURSE: The new methodology developed for the synthesis of benzoanthracenes has been broadened and extended to prepare more complex examples of polynuclear aromatic hydrocarbons. These have included highly alkylated benzanthracenes and benzanthracene derivatives capable of exhibiting optical activity. Functionalized benzanthracenes with the potential for conversion into other polynuclear aromatic hydrocarbons have been synthesized. Known and hypothetical oxygenated metabolites of some of these hydrocarbons have been prepared. Finally heteroaromatic polynuclear hydrocarbons have been synthesized.

It is proposed to continue the development and refinement of new synthetic approaches to polynuclear aromatic hydrocarbons and their metabolites such as arene oxides and hydroxylated derivatives.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polynuclear aromatic hydrocarbons are recognized to be of major environmental importance due to their widespread ecological distribution and, in some cases, their concentration in the food chain. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit critical biological and toxicological studies. Synthetic availability of appropriate model compounds allows further examination of the mechanisms of their biological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30041-07 LEC

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Hydrophobic Haptens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.W. Albro	Research Chemist	LEC NIEHS
Other:	K. Chae	Chemist	LEC NIEHS
	J.D. McKinney	Supervisory Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

Toxicological Research and Testing Program, NTP
Animal Husbandry Section, CMB

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No additional work will be initiated for immunoassay methods development for compounds of environmental interest. Project is being terminated due to change in research priorities.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques: condensation reactions, amination, reduction, mixed ester formation, active ester formation, convergent synthesis, spectroscopic and chromatographic characteriztion. Conjugation techniques: diazo coupling, mixed anhydride acylation, active ester acylation. Characterization of conjugates by UV spectroscopy, gel filtration, amino acid analysis, NMR spectrometry, and chemical assay of functional groups. Antibody production methods: standard procedures, with adjuvant, in rabbits. Antibody assay methods: double immunodiffusion, fluorescent antigen, radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: Work continues on the development of an immunoassay for DDE, a ubiquitous metabolite of DDT. This will be the last compound of environmental interest for which immunoassay methods will be developed intramurally. Project is being terminated due to change in research emphasis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Albro, P.W., Luster, M.I., Chae, K., Clark, G. and McKinney, J.D. Radioimmunoassay of Chlorinated Dibenzo-p dioxins. In Colowick/Kaplan: Langone/Van Vunakis: Methods in Enzymology, Vol. 84: Immunochemical Techniques Part D.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30050-06 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemical and Enzymatic Conjugation of Glutathione with Arene Oxides and Epoxides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	O. Hernandez	Visiting Scientist	LEC NIEHS
OTHER:	J. Bend	Research Pharmacologist	LP NIEHS
	A. Bhatia	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. NO1-CP-33387. Supplemented by NIEHS)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The chemical conjugation of specific benzo[a]pyrene oxides and other epoxides to glutathione, enzymatically and non-enzymatically, is being investigated using nuclear magnetic resonance (NMR) spectroscopy, chemical synthesis, and high pressure liquid chromatography (HPLC). The regiospecificity and stereospecificity of the conjugation reaction is being determined. A HPLC assay has been developed for the glutathione conjugates of benzo[a]pyrene 4,5-oxide and the conjugation with various purified glutathione s-transferases examined for stereochemical detail. The stereochemistry of the isomers of the glutathione adducts of styrene oxide and benzo[a]pyrene 4,5-oxide has been established.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy; high pressure liquid chromatography (HPLC); organic synthesis, in vitro biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: The absolute stereochemistry of the glutathione adducts of (+)-benzo[a]pyrene 4,5-oxide was established by using enantiomerically pure oxide(s) as substrate for the alkylation reaction. In general, the glutathione transferases show a preference for the oxirane carbon with the R configuration. This stereopreference is not restricted to (+)-benzo[a]pyrene 4,5-oxide but has also been detected with (+)-benz[a]anthracene 5,6-oxide and the pro-chiral substrates pyrene 4,5-oxide and phenanthrene 9,10-oxide. It is of more than casual interest to observe that this stereopreference is complementary to that of the epoxide hydrolase, another key detoxifying system, which shows stereoselectivity for the S configuration oxirane carbon.

The proposed course of action is to determine the absolute configuration of the glutathione conjugates described above and to characterize the glutathione adducts of (+)-Benz[a]anthracene 5,6-oxide.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanistic aspects of the GSH transferase reaction underscore the relevance of stereochemical factors in influencing the rate of elimination of enantiomeric oxides via the glutathione pathway.

PUBLICATIONS

Hernandez, O., Foureman, G.L., Cox, R.H., Bend, J.R., Walker, M., and Smith, B.R.: Stereo- and regioselectivity in the enzymatic conjugation of glutathione with (+)-benzo[a]pyrene 4,5-oxide. International Symposium on Polynuclear Aromatic Hydrocarbons, Battelle Laboratories, Columbus, Ohio, 1981, pp. 667-674.

Yagen, B., Hernandez, O., Bend, J.R., and Cox, R.H.: Synthesis and relative stereochemistry of the benzylic thioether diastereomers formed from glutathione and styrene oxides. Bioorg. Chem. 10, 299-310 (1981).

Hernandez, O. and Bend, J.R.: Metabolism of Epoxides. In Enzymatic Basis of Detoxication, W.B. Jakoby (ed.), Vol. 3, Academic Press, Inc., New York, 1982, pp. 207-228.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30051-06 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Specific Binding Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James D. McKinney	Supervisory Research Chemist	LEC NIEHS
Other:	P.W. Albro	Research Chemist	LEC NIEHS
	Kun Chae	Research Chemist	LEC NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The guinea pig was used as an extremely sensitive animal model to investigate the toxic effects of polybrominated biphenyl (PBB) mixtures and individual isomers and congeners. A human plasma protein has been shown to bind specifically to toxic planar/coplanar halogenated hydrocarbons of the dioxin type.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic methods along with X-ray crystallography and other methods for measuring physical/chemical properties with associated equipment and techniques was used primarily in this phase of the work. Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy using specifically labled (^{13}C, ^{19}F, ^{2}H, etc.) compounds where possible for studying complex molecular interactions is also used when solubility is not a problem. Isolation and characterization of specific binding site(s) in body tissue and fluid using standard biochemical methods, and modeling of receptor proteins as possible and required.

MAJOR FINDINGS AND PROPOSED COURSE: The guinea pig was used as an extremely sensitive animal model to investigate the toxic effects of polybrominated biphenyl (PBB) mixtures and individual isomers and congeners. Lethality of the mixture in guinea pigs appears to be associated with toxic coplanar molecular conformers of certain favorably substituted biphenyl components. The suspected isomers are being synthesized for individual testing in the guinea pig to complete the work. Toxicity of this type is similar if not identical, to that observed for related planar halogenated aromatic hydrocarbon demonstrated to bind the dioxin receptor.

A human plasma protein has been shown to bind specifically to toxic planar/coplanar halogenated hydrocarbons of the dioxin type. The exact nature of this binding is being studied using x-ray crystallographic techniques and theoretical chemistry approaches. Other work will attempt to show the relationship of such binding to the mechanism of dioxin toxicity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

McKinney, J.D., Albro, P.W. and Hass, J.R. Some structural aspects pf chloran-eigenic Polychlrorinated Napthalenes Chemosphere 10(9), N15-N17.

McKinney, J.D. and McConnell, E.E. Structural specificity and the dioxin receptor. In Chlorinated Dioxin and Related Compounds. Impact on the Environment, O. Hutzinger, Ed. (Pergamon Press Ltd. Oxford, England) Vol 5, pp. 367-382.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30064-05 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mass Spectrometry Studies on Low Volatility Samples.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.R. Hass	Research Chemist	LEC, NIEHS
Other:	H.-J. Walther	Visiting Fellow	LEC, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A double-focussing mass spectrometer was upgraded by the addition of a fast atom bombardment ion source. The mass spectra of various polar compounds were obtained. The FAB technique in general gave more intense beams from polar compounds than the ones previously obtained with field desorption.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Field desorption mass spectrometry/fast atom bombardment mass spectrometry (FAB).

The replacement of the source housing on the VG 7070 mass spectrometer with one equipped to do fast atom bombardment MS has led to a decreased use of the field desorption ion source on the ZAB-2F mass spectrometer. The FD source has been modified to enable field ionization kinetic studies. The use of FAB has enabled the observance of protonated molecular ions from di-and tri-nucleotides, nucleotide-benzanthracene adducts, glutathione conjugates, sulfate adducts with aflatoxins, peptides, and phosphatidyl choline esters.

The present limitation of the FAB system is the mass range of the 7070 mass spectrometer (currently 750 amu at 4kV acceleration voltage and 1300 amu at 2kV acceleration voltage). Current plans include upgrading the 7070 to an extended range instrument of mass range of approximately 2000, and construction of a FAB source for the ZAB-2F mass spectrometer.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Finding mass spectrometric methods for the analysis of small polynucleotide-carcinogen adducts and other small molecule/biopolymer adducts will permit a rapid method for identification of these compounds, permitting studies of the effects of neighboring groups upon the reactivity of the site attacked. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30065-05 LEC

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Mass Spectral Reactions in Field-Free Regions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.R. Hass	Research Chemist	LEC NIEHS
OTHER:	J. Yinon	Visiting Scientist	LEC NIEHS

COOPERATING UNITS (if any)
M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC.

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A detailed study of the mass spectrometry of the explosives RDX and HMX has resulted in a detailed knowledge of their fragmentation reactions under various ionization conditions.

Selective reaction monitoring has been demonstrated to be a useful technique for the confirmation of 2,3,7,8-TCDD in air filter samples.

The analysis of Firemaster FF-1 for minor and suspected toxic impurities has been accomplished using HRGC-HRMS.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: A collisional induced dissociation (CID) study of the explosives RDX and HMX was done using mass analyzed kinetic energy spectrometry (MIKES). High resolution mass spectra and MIKE-CID spectra of RDX and HMX were recorded in electron impact (EI), chemical ionization (CI) and negative chemical ionization (NCI).

Fragmentation pathways of the investigated compounds were determined in all three modes of ionization. It was found that a major part of the fragment ions in RDX and HMX originate from formation of the adduct ions $(M+NO)^+$ and $(M+NO_2)^+$ in EI and CI and $(M+NO_2)^-$ in NCI, followed by dissociation.

Collision induced decomposition reactions of the 2,3,7,8-tetrachlorodibenzo-p-dioxin molecule have been employed as a confirmatory technique. The primary screening method employed was high resolution exact mass measurement/high resolution gas chromatography. The confirmation of suspected positive samples involved monitoring the loss of Cl or COCl from the molecular ion, coupled with capillary gas chromatography.

The analysis of Firemaster FF-1 (or BP-6) for minor and suspected toxic impurities has been accomplished by high resolution GC-Selected Ion Monitoring at medium and high resolution, the mass spectrometer being operated under a computer controlled peak matching system. Analysis for polybrominated napthalenes reveals the presence of various components having an exact mass corresponding to the elemental compositions of tetra-, penta- and hexa-bromonapthalenes. The search for the corresponding polybrominated methylnapthalenes or methylbromonapthalenes results in a series of signals that have similar but incorrect exact masses. The analysis of the flame retardant mixture has also been extended to the measurement of the exact mass of some monochloropentabromobiphenyl isomers and to the screening for tetrabromobiphenyl isomers.

On-going projects include:

1. Comparison of quantitation of levels of 2,4,5-trichlorophenol and hexachlorobenzene in serum and urine samples using both high resolution exact mass measurements and collision-induced decomposition reactions.

2. Efforts are on-going to enable the coupling of a short "guard" column to a fused silica capillary column. This will hopefully effect good separations while still allowing the injection of relatively crude samples onto the column.

The photoionization efficiency curves and appearance energies for $C_4H_8O_2^+$, $C_3H_6O^+$, $C_3H_5O^+$, $C_2H_5O^+$, $C_2H_4O^+$, $C_2H_3O^+$, CH_3O^+, CH_2O^+, CHO^+, and $C_2H_4^+$ from 1,4-dioxane have been obtained. Structures and heats of formation for some of the ions are proposed. Fragment ion masses $44(C_2H_4O^+)$, $45(C_2H_5O^+)$ and $58(C_3H_6O^+)$ are the lowest energy dissociation products which appear at a

photon energy of about 10.5 eV. All three ions are produced from long-lived (metastable parent ions up to an energy of about 11.2 eV. The decay rates of internal energy selected parent ions were measured by photoion-photoelctron coincidence (PIPECO) and the results were compared to statistical theory (RRKM/QET) calculations. The three dissociation channels were found to be in competition with each other, and the data indicate that the dioxane ion does not isomerize to a more stable structure prior to dissociation. Kinetic energy release associated with the production of the m/e 58 fragment was measured as a function of the parent ion internal energy and found to be somewhat greater than that predicted by the statistical theory.

Photoionization efficiency curves and appearance energies for $C_4H_8O_2^+$, $C_3H_5O_2^+$, $C_4H_7O^+$, $C_2H_4O_2^+$, $C_3H_7^+$, $C_3H_6^+$, $C_2H_5^+$, and $C_2H_4^+$ from butanoic acid have been obtained. Structures and heats of formation for some of the ions are proposed. No evidence for metastable $C_4H_8O_2^+$ ions forming $C_3H_5O_2^+$ (m/e 73) and $C_2H_4O_2^+$ (m/e 60) was found in the photoion-photoelectron coincidence (PIPECO) results. An upper limit to the parent ion lifetimes is 0.1 μs. In contrast to the photoionization results, electron impact ionization produces strong $C_4H_8O_2^+$ metastables with lifetimes in the 10-μs range. This paradox can be resolved by postulating that photoionization produces only rapidly dissociating butanoic acid ions, while a long-lived isomerized structure (or structures), such as the enol form, are accessible by electron impact.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry (in combination with chromatography) is the most sensitive and specific analytical method presently available for dealing with complex samples of environmental/biological origins. The results of this project will increase our understanding of the fundamentals of the technique as well as provide more specific information for qualitative or quantitative analysis.

PUBLICATIONS

Harvan, D.J., Hass, J.R., Schroeder, J.L., and Corbett, B.J.: "Detection of tetrachlorodibenzodioxins in air filter samples". Analytical Chemistry, 53, 1755-1759, (1981).

Harvan, D.J., Hass, J.R., and Wood, D.: "Exact mass measurement with a computer-controlled peak matching system". Analytical Chemistry, 54, 332-334, (1982).

Harvan, D.J., Nystrom, J.A., Grady, W.L., Voyksner, R.D., Cerny, R.L., Bursey, M.M., Siegel, M.W., Yinon, J.Y., and Hass, J.R.: "Comparison of low and high energy collision induced decompositions of selected methyl ketones", Analytical Letters, 14, 985-993, (1981).

Fraser-Monteiro, M.L., Fraser-Monteiro, L., Butler, J.J., Baer, T., and Hass, J.R.: "Thermochemistry and dissociation dynamics of state-selected $C_4H_8O_2^+$ ions. 1. 1,4-Dioxane". The Journal of Physical Chemistry, 86, 739, (1982).

Butler, J.J., Fraser-Monteiro, M.L., Fraser-Monteiro, L., Baer, T., and Hass, J.R.: "Thermochemistry and dissociation dynamics of state-selected $C_4H_8O_2^+$ ions. 2. Butanoic acid". The Journal of Physical Chemistry, 86, 747, (1982).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30066-05 LEC

PERIOD COVERED
October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Synthetic Methods for Polyhalogenated Aromatics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.D. McKinney Supervisory Research Chemist LEC NIEHS
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1		0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Selected brominated biphenyls are being synthesized and characterized for further toxicological study of the components of fire retardent PBBs. These include a penta and two hexabromobiphenyl compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents, functional group transformation. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: Efforts are continuing in the synthesis and characterization of toxic components of the fire retardent chemicals PBBs. A pentabromobyphenyl has been prepared and charecterized according to the literature. Related hexabromo congeners are being selectively prepared using photolysis of higher brominated biphenyls. Preparation of these materials will complete the synthetic support needed for the toxicological study of PBBs.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is still a great need for simple synthetic routes to many polyhalogenated aromatics. This project will, hopefully, give simple synthetic routes to many toxicologically interesting polyhalogenated aromatics which are known to persist in the environment.

RALTECH SCIENTIFIC SERVICES - Madison, Wisconsin 60616
(NIH-NO1-ES-7-2141)

TITLE: Analysis of PCB's and DDE in Human Body Fluids and Tissue

CONTRACTOR'S PROJECT DIRECTOR: D.L. Hughes

PROJECT OFFICER (NIEHS): J.D. McKinney, Ph.D., Chief, LEC

DATE CONTRACT INITIATED: September 30, 1977

CURRENT LEVEL (1 year): $93,862

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 1000 to 1200 samples per year of breast milk, formula, blood serum and placental tissue for polychlorinated biphenyls (PCB's) and 1,1-bis(p-chlorophenyl)-2,2-dichloroethane (DDE). The desired detection thresholds range from 0.5 to 50 ppb depending on the type of sample.

METHODS EMPLOYED: Gas liquid electron capture chromatography (EC-GC) and usual sample preparation, clean-up, extraction and lipid determination techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Using methods developed previously on the contract, a total of 5207 samples of milk, milk substitutes, blood serum or placenta tissue have been analyzed for PCB and DDE content. About 600 samples remain to be done to complete the work. This represents a substantially larger number of samples than originally anticipated and has resulted in time and cost overruns. The PCB content confirmation work by perchlorination techniques will not be done as a part of this contract work. Detailed method papers for milk and blood sample analyses have been documented and submitted for publication. Interpretation of the results and correlation with biological findings are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possible effect of transplacental and breast milk transfer of environmental contaminants from mothers to babies is an important and recurring epidemiological question. It has been shown that environmental contamination of breast milk occurs in the United States. Organohalogen pesticides and the polychlorinated biphenyls (PCB's) are widespread contaminants of breast milk. This study provides an integral part of an overall study of the possible widespread contamination of breast milk by environmental contaminants as PCB's and their effects on infant development and health.

SRI INTERNATIONAL - Menlo Park, California
(NIH-NO1-ES-79-0006)

TITLE: Application and Development of Procedures for the Analytical Determination of Environmental Chemicals by Radioimmunoassay

CONTRACTOR'S PROJECT DIRECTOR: Chozo Mitoma

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D., Research Chemist, LEC

DATE CONTRACT INITIATED: June 1, 1979

CURRENT ANNUAL LEVEL: $174,089

PROJECT DESCRIPTION

OBJECTIVES: (1) To evaluate the performance of radioimmunoassays developed at NIEHS: (2) to develop suitable procedures for the application of the specified assays to environmental samples; (3) to apply the specified immunoassays to the analysis of samples.

METHODS EMPLOYED: Double-antibody radioimmunoassay, organic solvent extractions, chromatographic cleanup procedures, statistical analysis of data.

MAJOR FINDINGS AND PROPOSED COURSE: Subsequent to having fully confirmed the published characteristics of NIEHS's RIA for chlorinated dibenzo-p-dioxins, SRI has partially completed its attempted confirmation of the published characteristics of the RIA for chlorinated dibenzofurans. SRI has thus far been unsuccessful in its attempts to develop a solid-state RIA for dioxins or to obtain a monoclonal antibody to the 2,3,7,8-tetrachloro isomers. Problems associated with application of the RIA to soil samples have delayed the analytical work, but these problems are thought to have been largely overcome and analysis of soil samples is proceeding. If time permits, the RIA for dioxins will be applied to a series of samples of human milk.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One major purpose in developing these assays is the enabling of small, clinical laboratories to analyze samples for trace levels of these halogenated aromatic compounds without a need for extremely expensive, highly sophisticated instrumentation. An essential preliminary step is the demonstration that the NIEHS-developed techniques can be reproduced in other laboratories.

SCIENCE APPLICATIONS, INC. - La Jolla, California 92038
(NIH-NO1-ES-8-2105)

TITLE: Analysis of Total Organic Chlorine and Bromine Residues in Human Body Fluids and Tissues

CONTRACTOR'S PROJECT DIRECTOR: J. Reed

PROJECT OFFICER (NIEHS): J.D. McKinney, Chief, LEC

DATE CONTRACT INITIATED: December 20, 1977

CURRENT LEVEL (2 years): $462,000

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 1500 to 2000 samples per year of breast milk, formula, blood serum, and tissue for total organic chlorine (TOCl) and bromine (TOBr) of whole fluid or wet tissue or for total soluble organic chlorine (TSOCl) and bromine (TSOBr) content of portions of extractable lipids. The desired detection thresholds range from 5-20 ng chlorine and 0.1-15 ng bromine/gm milk.

METHODS EMPLOYED: BioGel P-2 desalting followed by standard methods of sample preparation for neutron activation analysis (NAA).

MAJOR FINDINGS AND PROPOSED COURSE: Using methods developed previously on the contract, a total of 3723 milk and serum samples have been analyzed for TOCl and TOBr, 1044 milk and milk substitutes for total chlorine and bromine, and about 424 placenta samples for total chlorine and bromine. About 500 milk and serum sample remain to be analyzed to complete the work. No additional placenta samples will be analyzed. Detailed method papers for milk and blood analyses have been submitted for publication.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Otainment of accurate and reliable results that lead to meaningful interpretation of the transfer of PCB's and DDE from mother to child through placental tissue membranes or through breast milk requires the separation of organic bound chloride and bromine from inorganic chlorides and bromides prior to neutron activation analysis for TOCl, TOBr, TSOCl and TSOBr. This study integrates contract NIH-NO1-ES-7-2141 to provide a mass balance which indicates whether all the halides are accounted for by the PCB's and DDE. In this manner, the results of the contract for the analysis of PCB's and DDE in human body fluids and tissues can be evaluated. This study will help resolve the important epidemiological effects of possible transplacental and breast milk transfer of environmental contaminants from mothers to babies in the United States.

LABORATORY OF MOLECULAR GENETICS

THE LABORATORY OF MOLECULAR GENETICS
Summary Statement

The primary goal of the Laboratory of Molecular Genetics (LMG) is to understand the molecular mechanisms of both spontaneous and induced mutagenesis. The rationale underlying this effort is that detailed knowledge of the ways in which organisms cope with challenges to their genetic integrity is crucial to assessing the risk posed to humans by mutagenic environmental chemicals. To address this issue, both genetic and biochemical approaches are employed, often requiring the development of new systems of analysis, within the general context of a highly interactive group.

The LMG is currently organized around six lead scientists under the general leadership of the Chief, Dr. John W. Drake. In addition to providing overall direction for the LMG, Dr. Drake conducts investigations into various aspects of mutagenesis in the bacteriophage T4 system. For instance, the alkylating agent methyl methanesulfonate has been shown to operate through two distinct mechanisms, one dependent upon and the other independent of the viral error-prone repair system. The common food preservative bisulfite, which had been claimed to be mutagenic in T4 and other systems, has been shown to be mutagenically inactive in the T4 system. A number of claims concerning the mechanism of ultraviolet mutagenesis in T4 have been shown to be of doubtful validity, since the underlying measurements were artefactual. A number of mutants have been discovered that define hypermutability at certain very restricted DNA sequences, revealing the existence of a level of genetic instability greater than previously encountered anywhere at the "point mutation" level; at least some of these mutants arise within short homopolymer DNA sequences.

The group led by Dr. Michael Volkert uses E. coli genetics to investigate the consequences of DNA damage and its response to DNA repair. These studies focus on both regulatory and enzymatic functions of genes involved in metabolism of DNA damage. The approaches include: (1) a study of mutants which are suppressors of recF, a gene involved in both genetic recombination and the repair of DNA damage; (2) examining the effects of a unique recA mutation upon suppression of recF UV sensitivity; (3) the production of a set of ØX174-sensitive E. coli strains carrying various repair deficiencies in known genes, to be used in reactivation and mutagensis studies with single-stranded DNA; and (4) most recently, selecting E. coli mutants defective in the repair of DNA damage induced by alkylating agents.

The group led by Dr. Lynn Ripley has focused on the role of T4 DNA polymerase in frameshift mutagenesis. A specific spectrum of spontaneous frameshift mutations is observed at various positions in the T4 rII locus in the presence of the wild-type DNA polymerase, and this spectrum varies dramatically with mutant DNA polymerase alleles. These data demonstrate a major role for the polymerase in determining frameshift mutation frequency and specificity, and imply that the mechanisms responsible for frameshift mutations are diverse. The T4 frameshift mutants are currently being sequenced in order to obtain information on specificity and putative mechanisms. A second area of interest involves the mechanisms of action of several mutagenic agents in the T4 system, including hydroxylamine, 2-aminopurine and heat. These studies also provide insights into mechanisms of discrimination against errors at sites of DNA damage during the subsequent replication of the DNA.

The group led by Dr. Barry Glickman uses genetic approaches in E. coli to address questions of mutation frequency and specificity. Of particular interest for questions of mechanism are the studies on the specificity of spontaneous and induced mutagenesis using the lacI gene of E. coli. This system has permitted determinations of the mutagenic specificities of X-rays, ^{3}H-decay, UV light, methyl methanesulfonate and ethyl methanesulfonate, providing insights into their mechanism of action in the production of mutations. A second area of interest is the role of methylation-instructed mismatch correction in error avoidance. Several mutants that perturb this process have been identified, and their analysis suggests that this repair pathway contributes substantially to the faithful maintenance of genetic information. The possibility is being investigated that mismatch repair is inducible and is involved in avoiding the indirect mutagenesis associated with error-prone repair.

The observations made in the E. coli lacI system and in the T4 rII frameshift mutagenesis system have suggested that DNA secondary structures may be involved in the production of several types of mutations. Close examination of a large body of information in the literature, carried out jointly by Drs. Ripley and Glickman, have lent strong support to this concept, due to the identification of numerous classes of mutational events which can be explained on this basis. Models have been proposed which make predictions that can be examined by DNA sequence analysis of mutants from the lacI and T4 rII systems. If valid, this concept has major implications for both spontaneous and induced mutagenesis as well as for evolutionary processes.

The group led by Dr. David Mace investigates the chemistry of DNA damage in order to understand how changes in DNA bases affect discrimination mechanisms. Efforts focus specifically on the observation that heat induces the rearrangement of nucleosides so that new nitrogens become attached to the sugar, giving rise to "neonucleosides". Deoxyneoguanosine has been detected in DNA under certain conditions, and the base pairing properties of this compound are now being studied; the formation of this base may well be a major component of spontaneous "heat" mutagenesis.

The group led by Dr. Thomas Kunkel investigates the frequency and specificity of mutagenesis in vitro using defined DNA targets and purified replication and repair proteins. The major focus has been on the fidelity of the DNA polymerases themselves. The ability of several prokaryotic DNA polymerases to discriminate against base-substitution errors, both at the insertion step and the editing (proofreading) step, has been assessed in an amber mutant reversion assay with single-stranded øX174 DNA. Similar studies have been performed with eukaryotic DNA polymerases, as well as with DNA binding proteins. A forward-mutation system is now being developed to expand these studies to include frameshift mutagenesis.

The group led by Dr. Akio Sugino is investigating basic mechanisms of DNA replication. In vitro complementation assays have been established for the identification and characterization of proteins involved in bacteriophage N4 and Ø29 replication in prokaryotes and for yeast 2-μm plasmid DNA replication. These studies have led to purification of the N4-encoded DNA polymerase and DNA binding protein, Ø29 terminal protein and yeast DNA polymerase, single-strand DNA binding protein and primase. With the eventual goal of cloning the gene for replicative polymerases, mutants have been identified in cultured Drosophila melanogaster cells and in yeast, mutants which are resistent to the drug aphidicolon; Attempts are now being made to develop DNA transformation systems with these cells. Studies are

also in progress with an E. coli RNase H mutant to delineate the role of this enzyme in DNA replication. The gene has been cloned and sequenced. Finally, as a measure of genetic instability, the DNA sequence polymorphism of the major noncoding region of human mitochondrial DNA is being established using individual placental DNAs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60082-05 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Spontaneous Changes in DNA Primary Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Mace	Senior Staff Fellow	LMG	NIEHS
Other:	B. D. Price	Chemist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project investigates the possibility that deoxyneoguanosine residues in DNA (hyprothesized to be the G·C → C·G transversion pathway intermediate for heat mutagenesis in bacteriophage T4) can form a base pair with a deoxyguanosine residue on the opposing strand of a double helix. The formation of this novel base in polymeric and monomeric form is being studied. Its capacity to form a base pair with guanosine residues will be approached by both physical and biochemical methods.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Column chromatography is used to effect separation of the products of the reaction of a base and sugar phosphate. A variety of spectral, biochemical and chemical (including synthetic) methods are used to characterize these products. In addition, attempts are being made to relate the formation of these products to events that occur in DNA concommittant with depurination.

MAJOR FINDINGS AND PROPOSED COURSE: To date we have shown that a free base (such as might arise during depurination of dA or dG residues in DNA) can react with the "free" sugar phosphate to effectively "repurinate" the DNA. The principal product appears to be that formed by covalent attachment of the exocyclic aminio group to the sugar, giving rise to a "neo" nucleoside. In addition, there is some evidence that other ring nitrogens of the purine ring can react to give additional and quite novel nucleosides.

In addition to model compound studies, the formation and characterization of neoguanosine and neoadenine have received particular attention. Moreover, we have shown the formation of deoxyneoguanosine in DNA under conditions of depurination.

Work is continuing on the reaction as it occurs in DNA. In the future, we will also attempt to investigate the base pairing properties of these novel nucleosides in nucleic acid polymers particularly in regard to their possible properties as templates for DNA replication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The heat-induced G•C → C•G pathway could be a major contirubtor to the mutational load sustained by the mammalian genome. Thus, it seems important to determine if the hypothesized transversion pathway (involving a thermally induced migration of the glycosylic bond of deoxyguanosine) actually does occur and is able to form a base-pair with guanosine during the synthesis of DNA by T4 DNA polymerase. If the results indicate that deoxyneoguanosine is involved, investigations into the existence of this pathway and its repair will be pursued in mammalian cell systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60107-04 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Cloning and Sequence Analysis of Various Regions of the T4 Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Sugino Visiting Scientist LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The bacteriophage T4 is a powerful tool in the analysis of basic mechanisms of mutagenesis. The genetics of the rII region have been extensively used and inferences of particular mutagenic pathways can be made. With molecular cloning and DNA sequencing techniques we can now confirm certain inferred pathways. Cloned sequences will also aid in the biochemical analysis of certain T4 functions involved in fidelity and repair. The genome of bacteriophage T4 will be cloned by recombinant DNA techniques. First our efforts have been developing new techniques for cloning of T4 DNA without making C-containing DNA. The primary cloning vector is M13. The cloned sequences have been probed for rII regions of interest by DNA:DNA hydridization. These regions have been sequenced.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Purified T4 DNA is digested with restriction endonuclease TaqI. Vector M13 mp7 replicative form DNA is restricted by restriction endonuclease AccI. Both DNAs are annealed, ligated by DNA ligase and transformed by E. coli. Clones of interest is selected by plaque hybridization. Identified cloned DNA is sequenced by Sanger's dideoxynucleotides termination methods.

MAJOR FINDINGS AND PROPOSED COURSE: It is discovered that restriction endonuclease TaqI can digest T4 DNA, unlike other restriction endonucleases. The TaqI digest of wild-type and mutant T4 DNAs have been cloned into a M13 mp 7 vector. rII regions were identified by plaque hybridization and their DNA sequences have been determined by Sanger's dideoxy-termination methods.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Much of what we know of basic mechanisms of mutagenesis has been inferred from the genetic system of phage T4. The cloning and sequencing of certain mutants will allow us to verify proposed pathways of mutagenesis by environmental mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60108-04 LMG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of DNA Replication in Prokaryotes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
Other:	J. Arendes	Visiting Fellow	LMG	NIEHS
	P. Carl	Guest Worker	LMG	NIEHS

COOPERATING UNITS (if any)
Dept. of Biophysics & Theoretical Biology, Univ. of Chicago, Chicago, IL
Dept. of Molecular and Medical Microbiology, University of Arizona
Tucson, Arizona

LAB/BRANCH
Laboratory of Molecular Genetics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

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1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A) An in vitro replication system for bacteriophage N4 has been developed to study the mechanism of its DNA replication. The system mimics in vivo DNA replication. The DNA replication proceeds continuously from both ends of the DNA molecule as in vivo. By using this system as complementation assay, we have purified three DNA replication proteins to homogeneity and identified their functions.

B) The Guest Worker (P. Carl) has isolated a mutant which is deficient in RNaseH activity. It has been speculated that RNaseH removes RNA primer from "Okazaki Fragment". We have studied the RNaseH mutant genetically and biochemically. Also, we have cloned and sequenced the RNaseH gene from wild-type and RNase H⁻ E. coli to generate a much tigher mutant of RNaseH by in vitro mutagenesis.

C) An in vitro replication system for B. subtilis ϕ29 phage has been developed. This system mimics in vivo DNA replication and a protein primes DNA replication

PHS-6040
(Rev. 2-81)

Summary of work continued....

of protein-dNTP complex, we have partially purified such priming protein and studied extensively mechanism of initiation of DNA replication.

PROJECT DESCRIPTION

METHODS EMPLOYED: In vitro DNA synthesis system. Wild type and various DNA replication-negative N4 and ϕ29 phages and the crude extracts from those phage-infected cells. Conventional column chromatographies and velocity sedimentation for purification of DNA replication proteins. Standard cloning technique. Restriction endonucleases. DNA-DNA and colony hybridization. RNaseH assay using RNA-DNA hybrid. Electron microscope, agarose and polyacrylamide electrophoresis, and electrofocusing gel.

MAJOR FINDINGS AND PROPOSED COURSE: A) N4 DNA replication. Coliphage N4 contains a linear double stranded DNA genome of 72 kb pairs and a DNA dependent RNA polymerase in its virions. N4 DNA replication does not require the activity of E. coli genes dnaA, dnaB, dnaC, dnaE, dnaG and polA. Mutants in at least eight N4 cistrons affect N4 DNA replication. One cistron codes for the virion RNA polymerase required for the synthesis of N4 early RNAs. The products of three other cistrons are required for transcription of N4 middle RNAs which code for at least four functions involved in DNA synthesis (DO). Moreover, a study of the behavior of temperature sensitive mutants in the virion RNA polymerase suggest that it also plays a direct role in N4 DNA replication. We have developed an in vitro system to study the mode of initiation of N4 DNA replication. N4 infected cells are gently lysed and the soluble proteins are concentrated by ammonium sulphate precipitation. DNA synthesis in this extract is totally dependent on the addition of exogenous native N4 DNA. N4 heat denatured and other DNAs are poor templates. No activity is detected in extracts derived from N4 DO mutant infection. However, mixing of extracts from two DO mutants, in different cistrons, restores activity to nearly wild type levels. We are using this complementation assay to purify the N4 coded components required for in vitro DNA synthesis. In the course of these experiments, the cistrons corresponding to the N4 coded DNA polymerase and DNA-binding protein have been identified and purified to homogeneity. In vitro N4 DNA replication starts from both ends of the DNA molecule and proceedscontinuously as does in vivo.

B) E. coli RNaseH. 1) The E. coli rnh gene has been cloned in order to confirm the map location we have assigned to this gene. Such a clone is an overproducer of this important enzyme. 2) Using the cloned DNA we have determined nucleotide sequence of the gene. Based on such nucleotide sequence information, we will construct potential RNase H mutants which will be propagated on plasmids. By inducing recombination between host and plasmid rnh sequences we plan to produce strains carrying solely mutant rnh sequences. Alternatively RNase mutants will be selected by classical in vivo techniques of localized mutagenesis. 3) The rnh mutants will be characterized with respect to their growth and macromolecular synthesis. In particular we shall examine DNA synthesis in the mutants to see if we can find evidence for an increased number of Okazaki pieces bearing RNA primers or changes in the length of primers. 4) The new rnh alleles will be combined with mutants such as polAexl already known to affect the processing of Okazaki pieces to see if more severe defects in the processing are found in the double mutant.

c) ϕ29 DNA Replication. The Bacillus subtilis phage ϕ29 contains a linear, double-stranded DNA molecule about 18 kilobases in length. The unique features of the ϕ29 genome are that its DNA contains a short inverted six base pair-long terminal repetition and that a protein is linked covalently to the 5'-terminal ends

of DNA strands. The ϕ29 DNA terminal protein is essential for DNA replication. In order to study precise mechanism of ϕ29 DNA replication, we have developed *in vitro* DNA replication system from ϕ29 infected *B. subtilis*. This system is totally dependent on exogenously added ϕ29 DNA containing terminal protein and proceeds DNA replication from both ends of the DNA molecule. Furthermore, crude extract made from terminal protein minus ϕ29 infected cells does not support *in vitro* DNA replication even if ϕ29 DNA containing terminal protein is added. On the other hand, if some fractions from wild-type ϕ29 infected cell extract is supplemented, the crude extract support DNA synthesis as much as wild-type crude extract.

Using this system as complementation assay, we have partially purified ϕ29 terminal protein which is essential for its DNA replication. This protein catalyzes the reaction of formation of protein-dAMP complex in the presence of ATP and ϕ29 DNA. And ϕ29-induced DNA polymerase, then adds nucleotides phage using such protein-dAMP complex as a primer.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, particularly the importance of replication protein complex, and will provide a precise understanding of mutagenic mechanisms.

PUBLICATIONS

Arendes, J., Carl, P. and Sugino, A.: A mutation in the *rnh*-locus of *Escherichia coli* affects the structural gene for RNase H, examination of the mutant and wild type protein. *J. Biol. Chem.* 257, 4719-4722 (1982).

Kimura, J., Sugino, A. and Itoh, J.: *In vitro* DNA replication of *Bacillus subtilis* phage ϕ29. *Proc. Natl. Acad. Sci., USA* (1982) *in press*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60109-04 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of DNA Replication in Eukaryotes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
Other:	F. E. Wilson-Coleman	Staff Fellow	LMG	NIEHS
	J. Arendes	Visiting Fellow	LMG	NIEHS
	K. C. Kim	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

Dept. of Biochemistry, Molecular & Cellular Biology, Northwestern University, Evanston, IL

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A) In vitro replication system of yeast 2-μm plasmid DNA mimics in vivo DNA replication. Crude extract made from cdc8 mutant cells shows temperature-sensitive DNA replication of 2-μm plasmid in vitro. Using this system as a complementation assay, CDC8 gene product has been purified to homogeneity and found to be a single-stranded DNA binding protein. This protein interacts specifically to yeast DNA polymerase I which is a true DNA replicase. In the course of fractionation of in vitro DNA replication activity, a DNA primase activity has been detected, and particially purified. This DNA primase activity has been extensively characterized.

B) A lot of attempts have been made to develop DNA transformation system of Drosophila melanogaster cultured cells. This is a crucial for isolation of DNA polymerase α gene(s) from Drosophila melanogaster cells.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cell culture of Drosophila melanogaster. DNA transformation. Standard DNA cloning technique. Chemical and enzymatic DNA nucleotide sequencing methods. DNA-sythesizing crude extract from wild type and various cdc mutants of yeast Saccharomyces cerevisiae. Agarose and polyacrylamide gel electrophoresis, sucrose or glycerol density gradient sedimentation, and electron microscopy to analyze the products of in vitro DNA replication systems. Ethyl methane sulfonate mutagenesis using D. melanogaster cell line and yeast S. cerevisiae to isolate mutants which possess an altered DNA polymerase α and DNA polymerase of yeast, for mutant selection. Conventional column chromatographies and velocity sedimentation centrifugation for purification of DNA polymerases and other DNA replication proteins.

MAJOR FINDINGS AND PROPOSED COURSE: A) DNA polymerase α is mainly responsible for nuclear DNA replication. Isolation of DNA polymerase α mutants would be very useful for studying several aspects of DNA polymerase biochemistry, including the regulation of biosynthesis of the enzyme and its role in various cellular reactions such as DNA repair and recombination as well as DNA replication itself.

Recently, it has been shown that "aphidicolin" inhibits not only mitosis, but also in vivo DNA replication (particularly DNA polymerase α activity) in the sea urchin. Moreover, it has been shown that this drug also inhibits DNA replication in vivo and in vitro where DNA polymerase α might be involved in eukaryotes, including SV40, adenovirus, Drosophila nuclear DNA replication and yeast nuclear and 2 μm DNA replication.

This project focuses on the isolation of DNA polymerase genes from Drosophila. We have isolated aphidicolin-resistant DNA polymerase α mutant from a Drosophila melanogaster cell line and are continuing to try to isolate ts mutants from these mutants. Meanwhile, we have been trying to isolate DNA polymerase α gene using DNA (gene) transfer methods. B) Isolation of various DNA polymerase mutants in yeast: Although many ts mutants are already available in yeast and some have been identified as DNA replication mutants, none is a DNA polymerase mutant. Virtually all DNA polymerase activity in crude extracts from yeast is inhibited by aphidicolin, as is in vivo DNA replication.

The drug-resistant mutants have been isolated, and from these we will be able to isolate a subset of DNA polymerase ts mutants. Then using these resistant mutants, we will isolate the DNA polymerase gene and clone it in both E. coli and yeast by the following technique.

Assuming that drug resistance is dominant, nuclear DNA from the drug-resistant cells will be digested with various restriction endonucleases, and then linked to the yeast 2 μm-DNA vector or to the E. coli pBR322 vector; recombinants will be selected using conventional methods. In the case of E. coli, $polA^{ts}$, $polC^{ts}$ or $polA^{ts}$ and $polC^{ts}$ will be used as the host.

To select yeast recombinants containing the mutant DNA polymerase gene, total recombinants are grown on plates containing aphidicolin. To select E. coli recombinants containing the yeast DNA polymerase gene, total recombinants are

grown at restrictive temperatures. Finally, using radioactive antibodies against yeast DNA polymerase we can identify clones containing the yeast DNA polymerase gene. C) To understand the mechanism of DNA replication in eukaryotes, we have developed an in vitro DNA replication system of yeast 2-μm plasmid. This system mimics in vivo and starts DNA synthesis at the same site as in vivo and DNA synthesis proceeds bidirectionally. Some of the cell division cycle mutant crude extracts did not support significant in vitro 2-μm DNA synthesis under the restrictive condition. Particularly cdc8 mutant crude extract showed that in vitro DNA replication is temperature sensitive. By using this system as a complementation assay, cdc8 complementation activitiy has been purified to homogeniety from wild-type and cdc8 mutant cells. This activity has a single-stranded DNA binding ability and interacts specifically to yeast DNA polymerase I, which is a true DNA replicase in yeast. In the course of fractionation and reconstitution experiments of in vitro DNA replication activity, DNA primase activity has been identified and partically purified. Thus primase synthesizes oligoribo and deoxyribonucleotides on a single-stranded DNA. Yeast DNA polymerase I, but not DNA polymerase II, utilizes such oligonucleotides as primers and synthesizes DNA very efficiently. Meanwhile, many DNA replication mutants have been isolated in our laboratory and Dr. Dumas' laboratory at Northwestern University. The mutants which are deficient in DNA primase and other DNA replication protein activities will be identified by using our in vitro DNA replication system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:. These studies help to understand the complexity of DNA replication, and will provide a precise understanding of mutagenic mechanism.

PUBLICATIONS

Sugino, A., Kojo, H., Greenberg, B. D., Brown, P. O., and Kim, K. C.: In vitro replication of yeast 2-μm plasmid DNA. ICN UCLA Symposia on Molecular and Cellular Biology; The Initiation of DNA Replication, Volumn XXI (eds. D. S. Ray and C. F. Fox, Academic Press), p. 529-553 (1981).

Kojo, H., Greenberg, B. D., and Sugino, A.: Yeast 2-μm plasmid DNA replication in vitro: Origin and direction. Proc. Natl. Acad. Sci. USA 78, 7261-7265 (1981).

Arendes, J., Kim, K. C., La Bonne, S., Dumas, L. B., and Sugino, A.: Yeast 2-μm plasmid DNA replication in vitro: Single-stranded DNA binding protein restores the cdc8 defect. Proc. Natl. Acad. Sci., USA, 79, in press (1982).

Wilson-Coleman, F. E. and Sugino, A.: Yeast 2-μm plasmid DNA replication in vitro: Purification of DNA primase activity. Proc. Natl. Acad. Sci., USA, 79, in press (1982).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60111-03 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Role of Gene 43 DNA Polymerase in Frameshift Mutagenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS
	J. de Boer (After July 1)	Visiting Fellow	LMG	NIEHS
	B. W. Glickman	Expert	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DNA polymerase of T4 plays a major role in spontaneous frameshift mutation. Using the T4 rII genetic system for measuring mutation, we have identified a number of polymerase mutant alleles which strongly increase frameshift mutation frequencies. The spectrum of frameshift production is unique to the polymerase mutation. To date, genetic positions of frameshift mutations have been determined and correlated to the wild type DNA sequence. The existence of the potential for the formation of DNA secondary structure in one frameshift region suggests a novel DNA structural precursor to frameshift mutation. Our studies indicate that the mechanisms of frameshift mutation are diverse and that the fidelity of DNA polymerases for base substitution mutation can be substantially different from frameshift mutation.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulation of bacteriophage T4 utilizing mutants in the rII genes to measure mutation rates and frequencies, and mutations in the DNA polymerase gene 43 to alter fidelity of DNA metabolism. Definition of frameshift mutations in the T4 rII region requires the DNA sequencing of rII mutants. Cloning of T4 DNA is technically complicated because of the presence of modified cytosine in the DNA which inhibits the cutting of T4 DNA by most restriction enzymes. We are developing methods which will allow us to sequence a number of DNA mutants without transfering each mutant to a genetic background (containing four additional unlinked mutations) to provide DNA in which C is not modified. (See # Z01 ES 60107-04 LMG).

MAJOR FINDINGS AND PROPOSED COURSE: The T4 DNA polymerase plays a major role in frameshift mutation frequency and specificity. We intend to use this as a tool to learn more about the mechanisms of frameshift mutagenesis and the mechanisms by which DNA polymerases achieve fidelity in DNA synthesis. The genetic studies to date have characterized the frequency distribution of spontaneous frameshift mutants produced by a variety of polymerases within approximately 130 base pairs of T4 rII DNA. This DNA target is large enough to provide a variety of mutational targets and small enough to allow isolated frameshifts to be sequenced easily.

Our genetic analysis of the frameshift mutants and correlation to the wild type DNA sequence has revealed that each of the six polymerase alleles tested to date produces a unique spectra of frameshift mutations. The most fascinating spectrum was that of polymerase mutant tsL141. This allele is an antimutator for A:T-site transition mutations and for frameshift mutations in repeated A:T base pair sequences. However, this allele produces a strong mutator effect at several DNA sequences within a 30 base pair region of the DNA target that is quasipalindromic. The correlation of this mutator effect with this DNA sequence feature has led to the development of several new models for frameshift mutation. The theoretical basis of these models has been developed. In one model, the correction of mismatched regions of hairpin structures formed by the quasi-palindromes produces the mutation. The same mutations can also be produced by DNA strand switching by the DNA polymerase. A second model for enhanced frameshift mutations in palindromic regions suggests that it is due to a translocation efect of the enzyme. Intriguingly another polymerase allele having properties similar to tsL141 has already been suggested as defective in translocation from in vitro studies (Gillin and Nossal, JBC 251: 5219, (1976)).

Distinctions between these and other models will be possible upon obtaining the DNA sequences of the mutants produced by the various DNA polymerases. All of the DNA polymerases tested produced a substantial number of frameshift mutations in this quasipalindromic region of the DNA target, but they are genetically distinguishable, and hence not identical. This region may be a hot spot for frameshift mutations arising by a variety of different mechanisms.

While the DNA polymerase is clearly involved in the frameshift process it is not clear whether the mutations arise as a consequence of aberrant DNA synthesis

accompanying recombination or repair, or whether they arise primarily during DNA replication. Since these processes are not readily separated within the T4 system, our approach will be to identify other mutators for frameshift mutation. This approach will have the additional advantage of identifying, perhaps unexpected, determinants of frameshift fidelity that may act independently of the DNA polymerase. The T4 system will offer a number of advantages in this search. Any frameshift mutators identified can be easily mapped relative to most genetic systems. This will be important if their mutator property is the only phenotype. If the only mutators isolated are polymerase alleles this will not be a disappointment. No polymerases have yet been found which are altered only in their frameshift fidelity and not in their influence on base substitution mutation, we would like to find such a mutant. Furthermore, the well characterized polymerase mutants of T4 were isolated first on the basis of their temperature sensitive phenotype and only secondarily were they identified as fidelity mutants. Whether mutants isolated primarily on the basis of their fidelity might have different properties is not known.

The DNA polymerases, due to their unique specificity of frameshift mutation, offer an exceptional opportunity to explore the mechanisms of frameshifts mutation produced by mutagens. Intercalating agents may have very strong, and very specific binding modes to DNA, but the importance of these various bindings to the mutational process is not understood. We intend to approach the question of specificity, by asking whether it is the polymerase or the mutagen or both that determine frameshift specificity induced by these chemicals.

These studies will provide the ground work for identifying critical features of the DNA polymerase likely to be important in fidelity. The T4 DNA polymerase is readily studied *in vitro*, and the models developed from the genetic experiments and the sequencing can be used to develop an *in vitro* system for studying frameshifts. We hope to develop such a system in a way that the same DNA sequence can be studied in both *in vivo* and *in vitro* reactions hence improving our ability to correlate the actual *in vivo* events with *in vitro* model systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Frameshift mechanisms are not understood. General models have been proposed but no specific enzymology impinging on these mutations is well characterized. Frameshifts represent a particularly large fraction of "detected" spontaneous mutations in procaryotic systems. The reason for this is at least two-fold. First, frameshifts are efficiently detected compared to missense mutations. Frameshift mutations generate multiple missense codons after the frameshift and frequenctly generate a prematurely terminated peptide. Thus frameshift mutations can have an extremely deleterious effect in protein coding DNA sequences. Second, spontaneous frameshifts show a strong sequence preference. Genes carrying such hot spots show high spontaneous frameshift frequencies. The lysozyme gene of T4 and the *rII* genes of T4 have frameshift hotspots consisting of runs of A:T basepairs. The *lac I* gene of *E. coli* has a frameshift hot spot consisting of a tetranucleotide sequence repeated three times in the wild type sequence. Both addition and deletion frequencies are high in those hotspot sequences in which they can be distinguished.

Many frameshift mutations in protein coding regions of eucaryotic organisms including man may well be recessive because of their extremely deleterious effect. Thus, such mutations might contribute heavily to the genetic load of the population. The importance of frameshift mutation in non-protein coding sequences is unknown.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60112-03 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Mechanism of Mismatch Correction in E. coli.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. W. Glickman	Expert	LMG	NIEHS
Other:	G. Moessen	Summer Grad	LMG	NIEHS
	R. Schaaper	Visiting Fellow	LMG	NIEHS
	M. Skrzynski	Student Employee	LMG	NIEHS
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COOPERATING UNITS (if any)

None

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0.3	0.3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An error-avoidance pathway for the correction of mispaired bases has been identified in E. coli. According to the proposed mechanism, discrimination between the "correct" parental DNA strand and the "error-containing" daughter strand depends upon DNA methylation. The E. coli genes dam, mutH, mutL, mutS and uvrD/E are thought to be involved. This DNA repair system may contribute to the high level of replicational fidelity observed in living organisms. Recent evidence suggest that the mismatch repair pathway is inducible and that mismatch repair can avoid indirect mutagenesis due to the induction of SOS repair.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Our approach has relied on standard mutagenesis analysis as well as the analysis of mutational specificity in the lacI gene of E. coli. Transfection experiments using heteroduplexed λ DNA were carried out to measure mismatch correction in wild-type and repair-deficient strains of E. coli.

MAJOR FINDINGS AND PROPOSED COURSE: In recent years a major error-avoidance pathway responsible for the removal of misincorporated bases during DNA replication has been identified. The mechanism involves the recognition and excision of mismatched bases and differentiates between the "correct" parental and error-containing daughter DNA strands on the basis of DNA methylation levels, and key to discrimination being that parental DNA strands are fully methylated and daughter DNA strands are, following DNA replication, non-methylated. Mutants of this error-avoidance pathway which have been characterized are the dam mutant, defective in DNA methylation with the resulting loss of strand discrimination, and the mutator mutants mutH, mutL and mutS which control an early step in mismatch base excision, probably incision itself. Genetic characterization of these mutants shows that they, along with uvrE, uvrD and recL, belong to the same repair pathway. The level of mutagenesis in a multiple mutant is the same as in a mutH mutant and is about 10,000-fold higher than in the wild-type strain. This suggests that the total error-rate in E. coli, about one error in 10^{10} nucleotides incroporated, is the result of dual processes involving proof-reading by the DNA polymerase itself (with an error-rate of about 10^{-6}) and post-replicative, methylation-instructed mismatch correction which reduces errors by another factor of 10^4. These two mechanisms alone might account for the high fidelity of DNA replication observed in living systems.

We have examined the influence of mismatch repair on the directionality and specificity of mutagenesis by base analogs. The amber hotspots seen during 2-aminopurine mutagenesis are also evident in mutH strains suggesting that their prevalence above other sites are not related to mismatch repair. Studies with N^4OH-cytosine which shows and AT → GC preference are also underway in the mismatch repair strains.

The recent finding that mismatch repair is inducible and that mismatch repair can reduce indirect mutagenesis resulting from SOS repair has opened new areas of research. We are presently exploiting the loss of mismatch repair in mismatch repair mutants to investigate the extent and specificity of indirect mutagenesis. Moreover, using Mu:lac fusions we intend to investigate the regulation of mismatch repair.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The identification and analysis of this error-avoidance pathway will contribute greatly to our understanding of the mechanism by which the cell maintains such a high level of fidelity during DNA replication.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60113-03 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Mutagenesis in E. coli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. Schaaper	Visiting Fellow	LMG	NIEHS
	P. Dalldorf	Guest Worker	LMG	NIEHS

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to achieve a better understanding of how the cellular repair capacity, the nature and extent of the DNA damage and cellular metabolism interact to determine the biologically important endpoints of survival and mutagenesis. The work reported in this project involves the genetical and biochemical characterization of the DNA repair processes involved in error avoidance and error fixation. In particular, we have investigated the affect of dose on mutational specificity in order to learn more about what mutational events occur as different cellular repair capacities become saturated. This data not only will provide a basis for an improved understanding of the mechanism by which mutation occurs but will also lay the groundwork for a more accurate understanding of low-dose effects and their associated risks.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The determination of mutagenic specificity requires the isolation and characterization of hundreds of independently occurring mutations. The principles of the technique can be stated as follows: 1) the isolation of large numbers of independently occurring lacI$^-$ mutants; 2) the determination of nonsense mutations by suppression analysis; 3) separation of nonsense mutations into groups on the basis of localization by mapping data and 4) the correlation of each mutation with a specific mutational site by an analysis of the suppression pattern in strains carrying well characterized nonsense suppressors. This process requires the use of special techniques for the rapid detection and analysis of mutants on a large scale. In order to facilite the analysis, the lacI$^-$ mutations are isolated on F'prolac episomes in strains having a chromosomal deletion for these genes.

The initial screening for nonsense mutations, the mapping and the suppression analysis occurs by replica-plating mating techniques where the F' is transferring into the appropriate strains. The result of the analysis of the mutations is a spectrum of base substitutions obtained by the analysis of independently occurring mutations. This technique, although laborious, allows the precise determination of mutational events including the influence of the neighboring bases. Moreover, an analysis of frameshift mutagenesis has been made possible by the inclusion of a trpA reversion system.

We are also using DNA sequencing to determine the mutational specificity. This is accomplished by homogenotization of the lacI mutant on the F' with pCM1, a derivative of pBR322 carrying lacI.

MAJOR FINDINGS AND PROPOSED COURSE: X-rays: following a dose of 30kR the specific locus rate was 4.5×10^{-10} mutations per rad per gene copy per cell and the nucleotide substitution rate was 2.2×10^{-12} per rad. At the doubling dose (4kR) the mutational spectrum contained a greater fraction of transistions (85%) and fewer transversions (15%) that after 30kR which produced both classes with a similar efficiency. The possible effect of SOS repair on the specificity was further investigated among revertants were also noted in "SOS" on and "SOS" off cells. These data show that cellular repair can influence qualitative as well as quantitative aspects of radiation mutagenesis.

H^3-decay: The biological effects of tritium decay was examined in the lacI system. Both killing and mutagenesis were monitored. The mutational spectra were similar to those of γ-rays and changed with dose. Transitions predominated at low doses while transitions and transversions occurred with equal frequency at higher doses.

UV-mutagenesis: An analysis of the mutation spectrum for UV light showed that most if not all mutagenesis occurred at sites where pyrimidine dimers could be formed. Several mutational hotspots were correlated with secondary DNA structure. An analysis of the dose response for various sites in the spectra demonstrate that different hotspots respond with different kinetics: spectra then appear quite different at different UV-fluences.

Akylating agents: Preliminary experiments were carried out to determine the effect of cellular repair capacity and dose on the mutagenic and toxic effects of alkylating agents. The role of the recA, lexA, umuC, uvrA, uvrB, uvrC, recF, recL, uvrD and uvrE genes upon the repair of EMS and MMS induced damage was examined. In this way we were able to separate direct mutational effects and indirect effects: Moreover, we found that a functional uvrA, uvrB, uvrC repair capacity was required for the excision of ethylation but not methylation base damage. The alk mutation results in hypermutability by EMS and also alters the lacI MMS spectrum.

Initial mutation specificity experiments with EMS and MMS show: 1) The mutational spectre are dose dependent. For example, in the case of the wild-type strain a low dose of MMS increased the mutation frequency by a factor of 50 and resulted in about 30% amber and ochre mutants among the lacI$^-$ mutants. However, while doubling this dose increased the induced mutation frequency by a factor of two, of this mutant population only 4.5% were ambers and ochres. This demonstrates that the mutants produced at the higher dose were qualitatively different from those produced at the lower dose. 2) The mutational spectrum of EMS is primarily GC to AT base substitutions. In the uvrB and dam$^-$ strains, however, all four transversion events were uncovered at significantly high frequencies. The molecular basis for the altered mutational spectra for EMS in these repair-deficient strains is being further investigated. 3) The mutational spectrum for MMS in the wild-type cell differs from that caused by EMS and MNNG in that both transitions and transversions are detected. This strengthens the idea that MMS works by a mechanism distinct from that of other alkylating agents. The production of the full spectra for MMS at a broad range of doses would help to clarify this question, particularly if the mutational spectra for these agents in the repair-deficient strains is also ascertained. 4) The mechanism of mutagenesis by MMS is thought to involve both direct and indirect effects. However, we have found the dam$^-$ strain to be nonmutable by MMS. This suggests a role for DNA repair mechanisms in the fixation of MMS damage which was hitherto unsuspected.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will lead to an improved knowledge of how DNA repair systems affect the mutational response and assist in clarifying the molecular basis for mutagenesis. Moreover, data are being obtained which will enable the assessment of risk estimates to be made using knowledge of molecular specificity rather than solely on the basis of empirical extrapolation.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60129-02 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nucleotide Sequence Variability Surrounding Origin of Replication of Human Mitochondrial DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. D. Greenberg	Biologist	LMG	NIEHS
Other:	A. Sugino	Visiting Scientist	LMG	NIEHS
	C. F. Aquadro	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mitochondria, cellular organelles present in all known eukaryotic cells, contain a semi-autonomous genetic system -- a unique quality among metazoans. In mammalian cells, there is a marked conservation of genetic function and arrangement within the mitochrondrial DNA (mtDNA) molecule, which is completely and symmetrically transcribed, and encodes either proteins or the protein-synthesizing apparatus of the mitochondria. The remaining portion of the mtDNA contains the origin of DNA replication and the only two known origins of transcription. Moreover, there is economy of nucleotide base sequence within a species, although intraspecific variability does exist as seen by detailed restriction endonuclease analyses. Other investigators have located the greatest degree of polymorphism to the region which contains the replicational and transcriptional control sequences. It has been our goal to characterize this variability at the level of nucleotide sequence. We have cloned this polymorphic region of human mtDNA isolated from several different individual placentas, performed restriction mapping and hybridization analyses to define the precise origin of replication within the cloned segments, and the variable regions were conformed by determination of the DNA sequence.

PROJECT DESCRIPTION

METHODS EMPLOYED: The desired segment of mtDNA was a previously defined 3.0 Kb KpnI fragment. This fragment was isolated and ligated into a KpnI site of the chimeric plasmid pAD23 which contains pBR322 plus the HindIII D fragment of Adenovirus-2 DNA. Positive clones were identified by standard colony hybridization techniques with minor modification.

Restriction maps of these inserts were prepared by standard methods including single, double, and triple digestions, partial digestion, end labeling analyses and Southern hybridization. The origin of replication was defined by labeling and isolating "7S DNA" from the native mtDNA preparations on sucrose gradients. This small single stranded DNA represents a putative replication intermediate of mtDNA whose 5'-terminus is complimentary to the H-strand replication origin of the native molecule. Hence hybridization to restriction degests of the insert identified this sequence within the clones.

Clone fragments were prepared and nucleotide sequencing has been performed by the method of Maxam and Gilbert.

MAJOR FINDINGS AND PROPOSED COURSE: We have cloned the major noncoding region of human mitochondrial DNA (mtDNA) from eleven human placentas. Partial nucleotide sequences of five of these clones have been determined, and they share a maximum of 900 bases around the origin of H-strand replication. Alignment of these sequences with others previously determined has revealed a striking pattern of nucleotide substitutions and insertion/deletion events. The level of sequence divergence significantly exceeds the reported estimates of divergence in coding regions. Two locally hypervariable regions have also been defined. More than 96% of the base changes are transitions, and length alterations have occurred exclusively by addition or deletion of mono-or dinucleotide segments within serially repeating stretches.

This region of the mitochondrial genome, which contains the initiation sites for replication and transcription, is the least conserved among species, with respect to both sequence and length (Anderson et al., 1981; Walberg and Clayton, 1981). Despite this overall lack of primary sequence conservation, several consistencies appear among the available mammalian mtDNA sequences within this region. Interspecifically, a conserved linear array of characteristic nucleotide stretches exists which nonetheless differ in primary sequence among species. Intraspecifically, several conserved blocks of nucleotides appear among humans, within domains deleted from the mtDNA of other species. These observations are consistent with both a species-specificity of nucleotide sequence, and a preservation of the necessary genetic functions among species. This provides a model for the evolution of protein-nucleic acid interactions in mammalian mitochondria.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Several investigators have suggested that the rate of evolution of the mtDNA molecule exceeds that of the chromosomal DNA. It is not known whether this reflects intrinsic mitochondrial processes such as permitted or purposeful base substitutions, or a lack of response to externally generated base changes, ie. inefficient correction mechanisms responding to mutagenic events. As an approach to resolving

this question we must first define the nature of the intraspecific sequence variability. Conclusions concerning randomness or specificity of sequence polymorphism must await these results, with direct bearing on mechanisms of mtDNA diversification.

Furthermore, since this polymorphic region contains the control sequences for the initiation of replication and transcription, these results may have a bearing on these processes, illuminating the requirements for the corresponding enzymatic recognition sequences.

Hence these studies will contribute to the understanding of the complexity of DNA replication and gene transcription, and provide evidence concerning the mechanisms generating the sequence variability in the mammalian mtDNAs.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60130-02 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Isolation of E. coli Mutants Defective in Repair of Alkylated DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Volkert Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of enzymatic processes have been identified in recent years which act to repair lesions in DNA induced by alkylating agents. These enzymes include the glycosylases, Apurinic/Apyrimidinic endonucleases and the methyltransferases. The biological consequences of defects in these enzyme systems are for the most part unknown. Few mutants have been identified which are defective in these processes. Two methods will be used to select for mutants defective in repair of alkylation damage to DNA. One will be selective for mutants unable to repair λ bacteriophage treated with alkylating agents. The second will be to mutagenize with the insertion mutagen Mu d(Ap lac) and screen directly for sensitive mutants. By these methods, we expect to accumulate strains containing mutations in the enzymes responsible for repair of alkylated DNA as well as mutations in genes which regulate these enzymes or are required for their expression. Such mutants will permit the systematic evaluation of the relative roles these repair enzymes perform in the recovery from alkylation damage, evaluation of the mutagenic properties of the lesions upon which specific enzymes act and identification of the genes which code for these enzymes.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic and microbiological techniques will be used for mutagenesis, mutant selection and isolation, and bacteriophage propagation.

MAJOR FINDINGS AND PROPOSED COURSE: Initially two methods will be used to obtain strains defection in the repair. The first will be a selective method to obtain mutants unable to repair bacteriophage treated with alkylating agents.

These will appear as mutants which are resistant to infection by bacteriophage treated with alkylating agents. While this procedure has the advantage that it is selective two major disadvantages are that some repair systems acting on bacterial DNA may not act on infecting bacteriophage. Mutations in genes of these DNA repair pathways will be missed; secondly, mutations in genes which have only a small effect on bacteriophage survival will likely remain undetected. Nonetheless, this technique will facilitate the isolation of mutants defective in the major pathways of repair of alkylation damage to DNA which act on damaged bacteriophage.

The second method will be to use the bacteriophage Mu d(Ap lac) as a mutagen, then screening for sensitivity to a variety of alkylating agents. The major disadvantage of this technique is that it is solely a screening technique and not selective also the insertion of the Mu d(Ap lac) phage into the chromosome may not be entirely random. The primary advantages are that insertion of this mutagen produces a selectable phenotype and when inserted in the proper orientation allows the determination of regulation of the gene into which it has inserted. When sufficient numbers of mutants sensitive to a variety of alkylating agents have been isolated the mutations responsible will be mapped and the strains grouped on the basis of potential gene identity. This will allow the identification of the genes involved in coding for the enzymes which repair alkylated DNA and the subsequent identification of their products, their in vivo effects on cell survival and mutagenesis and a better understanding of the mechanisms available to the cell for the recovery from alkylation damage to DNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will aid in our understanding of the metabolism of damaged DNA. How enzymes involved in the repair of alkylation damage to DNA act, what their effects are on the cellular level and how these enzymes are regulated at the genetic level.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60132-02 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interaction of recF143 and recA441 Mutations in DNA Repair and Mutagenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. R. Volkert	Senior Staff Fellow	LMG	NIEHS
Other:	M. A. Hartke	Biological Aid	LMG	NIEHS

COOPERATING UNITS (if any)

A. J. Clark, Department of Molecular Biology, University of California Berkeley, CA 94720

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SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mutations in the recF gene of E. coli cause a reduction in the expression of the SOS response, UV sensitivity, and a reduction in the level of induced recA protein synthesis. Increasing the levels of recA protein synthesis, either by introducing a multicopy plasmid bearing the recA gene or introducing a recA operator mutation, has no effect on the recF phenotype. Introduction of the recA441 mutation however, results in complex phenotypic changes which yield new insights into the physiological effects of recF mutations. The recA441 allele is unique since, unlike other recA mutations, it causes the expression of the SOS response without DNA damage upon incubation at 42°C.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic and microbiological techniques are employed for strain construction, genetic manipulation, cell survival and mutation assays.

MAJOR FINDINGS AND PROPOSED COURSE: We have now divided the effects of the recA441 mutation on recF143 mutant strains into two components. In recF mutants recA441 produces both a temperature dependent and temperature independent suppression of recF UV sensitivity. UV resistance is somewhat increased at 30°C and upon incubation at 42°C, the temperature at which the recA441 allele derepresses the SOS response constitutively, the UV resistance is greatly enhanced. In excision deficient derivatives of the various strains the temperature dependent component of suppression is absent whereas the temperature independent component remains. This shows that excision repair is at least in part a recF dependent pathway of repair. Previous experiments have shown that the excision dependent pathway of W-reactivation of double-stranded DNA phage (ie. inducible excision repair) is recF independent. However, other workers have shown that recF does block one type of excision, the long patch pathway of excision repair. Our experiments suggest that this pathway may be restored in recF mutants as a result of the recA441 mutation upon incubation at 42°C.

The nature of the temperature independent component of suppression of UV sensitivity is not completely understood. Our present experiments are designed to determine if this component is a recombinational pathway of DNA repair.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In wild type cells genetic damage can be metabolized in a variety of ways by a wide variety of enzymatic processes. The end points of these processes can be either restoration of the genetic material to its predamaged state mutational alteration of the DNA, either as a result of the damage or the repair, or cell death due to the inability to replicate or repair the damaged DNA. This study focuses on the regulatory and enzymatic properties of several genes, recA, recF and uvrA, which are involved in the metabolism of DNA damage and which affect the end result that such damage has on the cell. The elucidation of the metabolic activities of these genes will aid our understanding of the metabolism of DNA damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60133-02 LMG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Bisulfite Mutagenesis in Bacteriophage T4

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Drake	Chief, LMG	LMG	NIEHS
Other:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

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Laboratory of Molecular Genetics

SECTION

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bisulfite, a common food preservative, has been previously reported to be mutagenic in a number of cellular systems and also in phage T4. Because phage T4 employs 5-hydroxymethylcytosine instead of ordinary cytosine in its DNA, however, and because 5HMC is chemically far more resistant to the mutagenically significant action of bisulfite normally observed with cytosine itself, the action of bisulfite upon T4 should be critically re-examined. The earlier T4 report has now been shown to be irreproducible. Furthermore, bilsulfite is not even mutagenic for bacteriophage T4 whose 5-HMC has been largely replaced by ordinary cytosine, even when the treated phages are grown on host cells unable to excise uracil residues from DNA.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The induced reversion of well characterized T4 rII mutants is tested with bisulfite under a wide variety of conditions in order to determine whether bisulfite is at all mutagenic in ordinary T4 particles. Using a special strain carrying multiple genetic defects in 5HMC biosynthesis, T4 particles are also prepared containing cytosine instead of 5HMC in their DNA, and the bisulfite-induced revertibility of rII mutants is then examined in these particles. Where necessary, cellular hosts are used that are unable to repair bisulfite-induced premutational lesions.

MAJOR FINDINGS AND PROPOSED COURSE: The major result is the irreproducibility of a previous claim of bisulfite mutagenicity in T4. Surprisingly, bisulfite is also nonmutagenic for particles that contain cytosine instead of 5HMC in their DNA, even when the treated phages are plated on host cells unable to excise uracil from DNA. This work is now being prepared for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Bisulfite is a putative mutagen which, paradoxically, is used as a food preservative. Its mutagenic potency and specificity should therefore be determined as a part of the process of estimating its potential risk to humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60134-02 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Methylmethanesulfonate Mutagenesis in Bacteriophage T4

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. W. Drake Chief, LMG LMG NIEHS

COOPERATING UNITS (If any)

None

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Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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0.05	0.05	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

MMS in an alkylating agent with a distribution of preferred sites of methylation of nucleic acid bases quite different from that of several other monofunctional methylators, and in cellular systems MMS has always required the operation of an "error-prone repair system" to be mutagenic. In phage T4, however, MMS displays a dual mechanism, acting partly through the viral WXY error-prone repair system and partly independently of that system. WXY-dependent mutagenesis produces frameshifts and a variety of base-pair substitutions, as do other mutagens acting through this system (such as UV and gamma radiations). WXY-independent MMS mutagenesis produces mutations only at G:C base pairs, and these probably consist of transitions. The second mechanism is consistent with the observed (but infrequent) guanine-O^6 methylation by MMS, and the differences between phage T4 and cellular systems is consistent with a progressive increase in DNA polymerase discrimination associated with progressive decreases in mutability with increasing genome size.

PROJECT DESCRIPTION

METHODS EMPLOYED: MMS-induced reversion is measured in T4 rII mutants previously closely characterized as to available paths of reversion; and in a converse approach, MMS-induced rII mutants are characterized for revertibility by mechanism-specific diagnostic mutagens such as proflavine, 2-aminopurine, 5-bromouracil and hydroxylamine. MMS-induced forward and reverse mutation is also carried out in genetic backgrounds defective in WXY-mediated error-prone repair, and in other genetic backgrounds likely to give information about induced mutagen specificities.

MAJOR FINDINGS AND PROPOSED COURSE: The major finding to date has been that MMS operates through two distinct mechanisms, one requiring error-prone processing of premutational lesions and the other acting independently of this system. This line of work is nearing completion and will be published soon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The monofunctional methylating and ethylating agents have been powerful and highly instructive models for understanding chemically induced mutation and carcinogenesis. The mutagenic properties of MMS as displayed in phage T4 may provide insights into the mutagenic process in more complex mammalian systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES-60135-01 LMG

PERIOD COVERED

October1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Instability in Bacteriophage T4

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. W. Drake Chief, LMG LMG NIEHS

COOPERATING UNITS (if any)

None

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Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A class of bacteriophage T4rII mutants was observed some years ago, in a collection induced by gamma irradiation, that reverted at very high rates; stocks could contain on the order of 1% of revertants. Contrary to expectations based on previous work in other organisms, as well as in T4, these mutants did not contain duplications of substantial length, but instead behaved like point mutants, or at least mutants comprising no more than a few base pair changes. A much larger collection of such mutants will be sought among spontaneous and gamma-induced rII mutants, and will be subjected to genetical analysis to determine their nature. Eventually an attempt will be made to determine the mutant DNA sequences.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Forward r mutants will be screened in T4 stocks untreated or treated with about 500 KR of gamma irradiation. The unstable mutants will be identified by their high reversion rates. They will be subjected to reversion and recombination tests to characterize the behavior of their unstable sites, to estimate their physical extent, and to localize them sufficiently closely to make DNA sequence determination feasible.

MAJOR FINDINGS AND PROPOSED COURSE: It now appears that most or all of the unstable mutants detected among the survivors or gamma irradiation are components of the spontaneous background. Mutants have been discovered displaying a continuum of revertant frequencies from about 0.01% to about 5%. Their genetical properties are currently under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Genetic instability is receiving renewed attention because of its frequent association with transposable genetic elements, which in turn are important components of the spontaneous mutation rate in many organisms, including mammals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES-60136-01 LMG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ultraviolet Mutagenesis in Bacteriophage T4

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. W. Drake Chief, LMG LMG NIEHS

COOPERATING UNITS (if any)

None

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SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

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0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of published papers employ an ultraviolet-induced T4 reversion system to explore aspects of UV mutagenesis. Concern about a possible artefact in the measurements, plate reactivation, prompted measurements to determine the extent to which the putative reversion response is independent of the plating density of irradiated particles. All preliminary experiments indicate that most of the claimed reversion is a reactivation artefact. The experiments will now be repeated under conditions which more exactly reproduce those described in the published papers.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Stocks of T4 mutants of the rII locus or of a number of late-acting genes are ultraviolet-irradiated to a range of survivals and plated on host cells selective for revertants, at a variety of phage plating densities. In the initial experiments, plating conditions typical for this laboratory have been employed.

MAJOR FINDINGS AND PROPOSED COURSE: Increased frequencies of apparent revertants per surviving particle were readily observed with increasing UV doses, but most of this increase disappeared as the plating density of the irradiated particles on the selective host was decreased. The experiments will now be repeated using plating conditions (host cells, media) that duplicate those described in previous reports claiming that the system measures induced reversion.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our understanding of the mutagenic response via "error-prone repair" is based most strongly upon two systems, T4 and E. coli. UV is used because it is safer than most chemical mutagens, while acting in a similar manner. A number of published claims about the mechanism of UV mutagenesis in phage T4 are at stake because of the apparent operation of an experimental artefact.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES-60137-01 LMG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Induced Reactivation of UV Damaged øX174 Bacteriophage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Volkert Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DNA damage to E. coli elicits a response (the SOS response) which results in the derepression of at least 13 different genes or operons. One of these operons, the umuC operon produces two proteins which are required for the expression of induced mutagenesis. Current hypotheses state that the mutagenesis resulting from the derepression of the umuC operon performs a DNA repair function which allows DNA polymerase to by-pass UV lesions in DNA templates. UV damaged single-stranded DNA phage, such as øX174, can be reactivated by UV treatment of the host. The hypothetical umuC dependent DNA repair system has been proported to be responsible for this induced reactivation and mutagenesis of øX174.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Bacteriophage will be treated with UV light. UV damaged phage will then be used to infect øX174 sensitive host cells which either were or were not subjected previously to an SOS inducing treatment. Phage survival will be measured and induced reactivation values calculated. Mutagenesis of øX174 will be determined by scoring for reversion of am3 and am50 mutants of øX174 on Su^+ indicator cells.

MAJOR FINDINGS AND PROPOSED COURSE: A set of isogenic E. coli B/r strains have been constructed which are sensitive to øX174 bacteriophage. These strains carry several different mutations in various combinations which will allow the quantitative characterization of UV induced reactivation and mutagenesis of UV damaged øX174. The effect of the tif-1 allele of recA (also known as recA441) and umuC mutations on these processes.

The tif-1 allele of recA allows the expression of the SOS response without DNA damage to the host simply by incubation at 42°C prior to infection with øX174. We currently find a small increase in øX174 survival when this is assayed on a tif-1 mutant host which has previously been incubated at 42°C, indicating that some inducible repair active on øX174 is induced at this temperature. Current experiments are designed to determine if this small enhancement of survival comprises a portion of, or is additive to, UV induced reactivation of øX174, and whether both the temperature induced øX174 reactivation of the tif-1 mutant strain and the UV induced reactivation of both the tif-1 and wild type strain are lost when a umuC mutation is introduced.

The plasmid pKM101 carries a gene muc, which is analageous to the chromosomal umuC gene. Moreover, this plasmid enhances greatly both UV induced mutagenesis and UV-survival of cells in which it resides. Since the mechanism of DNA repair which is expressed by the pKM101 muc operon is believed to be similar to umuC, this plasmid would be expected to increase greatly induced reactivation and mutagenesis of øX174 as well as complement the defect in these processes when it is introduced into a umuC mutant. To answer these questions we have now completed the construction of E. coli B/r strains which are tif^+, tif-1, $umuC^-$ and $pKM101^+$, in all combinations.

E. coli B/r was used for these studies because of the large quantifiable effect of tif-1 on mutagenesis of the host bacterium and the greater ease and reliability of the host trp reversion mutagenesis assay over the E. coli K12 His reversion assay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pathway(s) of DNA repair which act on single stranded DNA phage are intimately associated with the production of mutations. This study is designed to probe that association both from a mechanistic and regulatory standpoint and aid in our understanding of how mutations result during the metabolism of damaged DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES-60138-01 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Suppression of recF Mutations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. R. Volkert	Senior Staff Fellow	LMG	NIEHS
Other:	M. A. Hartke	Biological Aid	LMG	NIEHS

COOPERATING UNITS (if any)

None

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Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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1.0	0.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The recF gene of E. coli plays a role in both genetic recombination and repair of DNA damaged by a variety of agents. Its role in these processes is presently not understood. We have selected for suppressors of recF (srf mutations) in a variety of genetic backgrounds. One class of revertants bear mutations linked to recA and restores both UV resistance and genetic recombination ability in a recB, recC, sbcB and recF. In this genetic background a large proportion of DNA repair and all genetic recombinations requires the recA and recF genes. We are currently trying to determine if these mutations are in fact mutant alleles of recA. If so, what recA protein changes have occurred which now allows recombination and repair to proceed in the absence of either wild type recBC or recF activities.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic and microbiological techniques are employed for strain construction, genetic manipulation, cell survival and mutation assays.

MAJOR FINDINGS AND PROPROSED COURSE: Our first result is that it is possible to restore UV resistance and recombination proficiency to strains which lack both the RecBC and RecF pathways of DNA repair and recombination. We have divided these mutant strains into two subgroups: 1) Those whose srf-mutations lie in or near recA and 2) those whose srf-mutations lie elsewhere on the chromosome. To date we have concentrated only on those srf mutations which lie in or near recA which we call srfA.

Our results demonstrate that mutations in or near recA can restore repair proficiency to a strain carrying mutations in recB recC sbcB and recF genes. The result that recombination proficiency is also restored by srfA mutations suggests that the increased UV resistance is due, at least in part, to increased recombinational repair activity. We will examine this more carefully by determining the ability of these strains to join daughter strand gaps, a measure of postreplicational recombination repair.

srfA mutations have been introduced into other genetic backgrounds in order to demonstrate two important properties. 1) recB recC sbcB mutations are not required in order for suppression to occur since recF143 single mutant strains are rendered UV resistant by srfA mutations. 2) srfA mutations are not allele specific since recB21 recC22 sbcB15 mutant strains which carry a Tn3 insertion in recF (recF332::Tn3) also become UV resistant when srfA is introduced. Moreover, the result that recF insertion mutants are rendered UV resistant by srfA in strains which lack recBC enzyme activity raises several intriguing possibilities: either recA can be altered in such a manner that it can function independently of either recBC or recF gene products; recA can be altered to interact with a third gene product which can substitute for recF; or srfA has altered another gene product near recA which can substitute for recF. Our current efforts are focused upon distinguishing between the above possibilities and characterizing further the effects of srfA mutations and determining the exact nature of the srfA mutations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: recA and recF are two key genes involved in metabolism of normal and damaged DNA. An understanding of the nature of the interactions of these gene products will advance our understanding of the mechanisms by which enzymes involved in the process of genetic recombination function in repair or recovery from genetic damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60139-01 LMG

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Mutagenesis With Defined Replication Proteins in vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. A. Kunkel Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The proposed experiments are intended to provide basic information on the relative frequency and specificity of base substitution and frameshift mutational events and, more importantly, to address the question of the mechanisms through which these events are produced at the molecular level. Focus will be on studies with purified replication proteins including DNA polymerases. These will be used to synthesize defined viral DNA probes in vitro which will permit the selection of various mutational events in in vivo biological assays. The exact nature of the mutational events will be determined by DNA sequence analysis.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Two types of biological transfection assays for viral DNA will be employed. The first measures reversion of amber mutations in øX174 DNA and the second measures a spectrum of forward mutational events as well as defined reversion events in M13mp DNA. DNA polymerases with other purified replication proteins (purified by standard techniques) will be used to synthesize new DNA in vitro using the viral DNA as template. Mutants are then selected as wild type revertants of amber mutations (øX174) or based on the ability to produce β-galactosidase (M13mp). The øX174 assay system is currently in use and the M13mp assay is to be developed.

MAJOR FINDINGS AND PROPOSED COURSE: Both DNA polymerases and single-stranded DNA binding protein have a significant role in determining the frequency and specificity of base substitution mutagenesis. Discrimination mechanisms involve both the proper selection of bases for incorporation and the ability to excise infrequent incorporation errors. This latter function has to date been demonstrated to have a major role in fidelity for prokaryotic DNA polymerases only. One of the first experiments planned is to determine if proofreading contributes to the accuracy of DNA synthesis in higher organisms. Several eukaryotic DNA polymerases are available which contain potential proofreading activity, and the øX174 assay system is amenable to the analysis.

Because most in vitro studies have focused on base substitution errors yet other types of mutational events are frequent (e.g. frameshifts), we will develop an in vitro assay to detect a spectrum of mutational events in a forward mutational assay. In addition, to obtain information on the mechanisms(s) of mutagenesis we will develop an assay to revert specific types of mutations. Both assays systems are chosen to allow direct DNA sequence analysis of the mutational event. We plan to use specifically defined DNA targets, having different primary DNA sequences, neighboring nucleotide effects and putative secondary structure, combined with the use of specific replication proteins having different properties which affect discrimination against errors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The current gaps in our knowledge of both base substitution and frameshift fidelity determinants stem in part from the lack of a system to address questions of frequency and specificity in a manner which will allow a determination of the exact nature of these rare events and at the same time permit analysis of mechanisms at the level of protein-nucleic acid interactions. The proposed experiments are intended to provide such information, which is basic to understanding mutagenesis at the molecular level. A determination of the cellular mechanisms for achieving the accurate production and maintenance of the genetic information is essential to understanding several fundamental biological processes.

LABORATORY OF PHARMACOLOGY

LABORATORY OF PHARMACOLOGY
Summary Statement

The Laboratory of Pharmacology carries out research to elucidate the relationships between the transformation and translocation of chemicals and toxicity in various target organs and cells of the body. A multidisciplinary approach is used in these investigations: pharmacologists, biochemists, chemists, endocrinologists, pathologists, physiologists, toxicologists, mathematicians and statisticians participate. This laboratory provides a central focus at NIEHS for using pharmacological and pharmacokinetic concepts to characterize, in detail, the mechanisms by which environmental contaminants exert biological effects. It plans and conducts studies 1) to determine the metabolic basis for selective/specific damage to certain organs and cell types which is characteristic of some toxins, 2) to elucidate the mechanisms whereby chemicals with hormonal activity alter the normal functions of organ systems, 3) to determine the role of membrane structure and function in excretion and toxicity of pollutants, 4) to correlate organ and cell structure with function both before and after exposure to chemicals, 5) to identify sensitive biochemical, pharmacological, physiological and pathological indicators of target organ/cell toxicity useful for the early detection and prediction of toxicity in experimental animals and humans, and 6) to provide reliable end points for the extrapolation of dose-dependent chemical effects across animal species and to humans. The Laboratory of Pharmacology also serves as a focal point within NIEHS, NIH and DHHS for marine and freshwater biomedical research. In this context we are especially interested in possible direct impact on human health by contaminants present in the aquatic environment (including drinking water) and accumulated by aquatic animals. Presently, the Laboratory of Pharmacology contains the following groups: Molecular and Comparative Pharmacology, Cell Pharmacology and Receptor Pharmacology.

A. Molecular and Comparative Pharmacology Group (Leader: Dr. J.R. Bend)

The overall activity of this group can be described as an integrated, multifaceted effort concerned with understanding the role of chemical metabolism, transport and excretion in the mediation of toxicity such as overt tissue damage, or more subtle effects such as carcinogenesis, mutagenesis, teratogenesis.

For many chemicals, the processes of metabolism are means of both activation and inactivation and the relative activities of these pathways/steps, as well as their location in different cells, and parts of cells, are most critical to the particular outcome of exposure to any given chemical. That these processes of metabolic activation and inactivation are themselves often controlled by genetics, as well as being affected by age, sex, disease and environment, further complicates the understanding of their role in the effects of any given chemical in any given tissue or animal species or individual of that species at any specific time of exposure.

Different approaches to the study and use of chemical-metabolizing systems are also used in this laboratory. For example, this group uses a variety of aquatic animals and mammalian species for comparison purposes to understand toxicological/pharmacological/physiological effects and problems. It has programs in North Carolina (at NIEHS), at the C. V. Whitney Marine Laboratory for Experimental Biology and Medicine, University of Florida (Gainesville), Marineland, Florida, and at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine (summer season only). Dr. Bend heads the programs at NIEHS and in Maine and Dr. Pritchard heads the off-site marine biomedical laboratory in Florida.

Major emphasis is currently focused on toxication-detoxication systems, transport and excretory mechanisms and membrane toxicity. The uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism in the storage and the chemical form of the accumulated xenobiotics in these species is assessed. The major emphasis is on how, why and where marine species accumulate pollutants which have potential for harm to man and whether or not mixtures of pollutants may lead to accumulation of more toxic forms or higher levels of pollutants than single chemical exposure. Effect of water temperature on metabolic, storage and excretion processes is being studied. Particular emphasis is being placed on studying formation and further metabolism of chemically reactive metabolites (e.g., arene oxides from polycyclic hydrocarbons). Special importance is also given to the cytochrome P-450-containing monooxygenase (MO) system and other hydrolytic and conjugating pathways, including effects of pre-exposure to environmental contaminants on these enzyme systems. Where biologically significant enzyme induction is observed, the induced system is characterized in considerable detail.

The factors which determine the rate of xenobiotic excretion are also evaluated in aquatic and mammalian species in detail to help assess the mechanisms leading to toxicity of chemicals that occur as environmental pollutants.

Another major purpose of this section is to serve as a national focus for an aquatic pharmacology/toxicology program -- to promote awareness of and use of such aquatic species and systems in better understanding human disease and contributions of pollution to such disease.

The collaborative efforts of this group demonstrate both its desire to share expertise where possible as well as to make use of the many opportunities for introducing more powerful and new approaches in this research area of chemical metabolism as related to toxicity. This group also interacts very closely with the Cell Pharmacology Group of Dr. J. R. Fouts.

Recent Accomplishments:

1. Dr. Bend's laboratory:

   a. The stereoselectivity of purified rat liver glutathione transferases was determined with ^{3}H-R- and ^{3}H-S-styrene 7,8-oxide enantiomers as substrates. Stereoselectivity was found to be a property of the individual glutathione transferase isozyme. Regioselectivity studies with racemic substrate demonstrated the preferential formation of one of the two possible positional isomeric products, depending upon the styrene oxide enantiomer.

   b. A method was developed which allows assessment of stereochemical preference in the cytochrome P-450-dependent oxidation of styrene.

   c. Rabbit pulmonary microsomes were shown to be about an order of magnitude more reactive, per nmole cytochrome P-450, than hepatic microsomes at converting 2-acetylaminofluorene (2-AAF) to oxidized metabolites. Microsomes prepared from Clara cells and alveolar type II cells isolated from rabbit lungs each metabolized 2-AAF to the same oxidative products formed by pulmonary microsomes; Clara cells were more active at catalyzing this reaction, consistent with their higher cytochrome P-450 content.

d. Five distinct glutathione transferases were isolated from the liver of the little skate, Raja erinacea. Four of these enzymes efficiently catalyze the reaction between glutathione and the K-region polycyclic arene oxides benzo(a)pyrene 4,5-oxide, pyrene 4,5-oxide, phenanthrene 9,10-oxide and benz(a)anthracene 5,6-oxide. The major transferase, E-4, was shown to be stereospecific with each of these substrates; that is only one of each possible pair of diasteromeric glutathione adducts is formed. With benzo(a)pyrene 4,5-oxide, enzymatic attack of the sulfur atom of glutathione occurs only at the carbon atom of the arene oxide having R configuration. The four "efficient" glutathione transferases show a strong enantiomeric selectivity for (-)-4R,5S-benzo(a)pyrene 4,5-oxide. The other enzyme, E-1, prefers the (+)-5S,4R-enantiomer. The ultimate carcinogen trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene was also shown to be a substrate for E-4 although its turnover was approximately two orders of magnitude lower than that of BP 4,5-oxide.

2. Dr. Philpot's laboratory:

Several important observations have been made regarding the effects of various compounds on the concentrations of cytochrome P-450 isozymes in lung and liver:

a. Treatment of rabbits with phenobarbital induces the synthesis of two isozymes of cytochrome P-450, forms 2 and 5, in the liver. These isozymes are minor forms in the livers of untreated rabbits but are major forms in lung.

b. Treatment with phenobarbital has no effect on the concentrations of forms 2 and 5 in the lung.

c. The synthesis of cytochrome P-450, form 6 in the lung is repressed by phenobarbital but induced by co-planar PCB isomers. The inductive effect of the PCBs is blocked by phenobarbital.

d. Phenobarbital induction of the synthesis of form 2 in the liver is blocked by PCBs.

3. Dr. Pritchard's laboratory:

a. Excretion of polycyclic aromatic hydrocarbons and their metabolites was shown to depend upon a) metabolism to glucuronide and sulfate conjugates and b) active tubular secretion of these conjugates via the organic anion transport system. Inhibition of organic anion transport by other anions, including the phenoxyacetic acid herbicides, produces marked retardation of elimination.

b. Sulfate homeostasis in both mammals and fish is the product of renal tubular transport. We have shown that in fish, where net secretion occurs, proton-coupled cotransport at the contralumenal membrane drives sulfate uphill into the tubular cell. This was the first demonstration of proton-coupled cotransport at the basolateral membrane. Exit of sulfate across the lumenal membrane is mediated via an anion exchanger. Bicarbonate was the most effective counterion. In mammals, reabsorption of sulfate predominates. The lumenal entry step was shown by others to be sodium-coupled. We have demonstrated that the countralumenal exit step is

mediated by an anion exchanger very similar to that of the teleost lumenal membrane. Not only do these results shown how anions are handled by both mammals and fish, but they also suggest a broader general importance of membrane bound anion exchange proteins in pH and volume regulation as well as transepithelial transport.

4. Dr. Anderson's laboratory:

   a. BP metabolite-DNA adducts were observed in lung, liver, forestomach, brain, colon, kidney and muscle of A/HeJ mice after an oral dose of ^{3}H-BP. Surprisingly, the specific activities (pmol/mg DNA) of the BP metabolite-DNA adducts did not vary more than 2-fold between these tissues. In contrast, there was more than a 20-fold variation between the tissues in binding of BP metabolites to protein. This data suggest that the metabolic capacity of a tissue might not be the rate-limiting step in the formation of BP metabolite-DNA adducts.

   b. BP-induced DNA repair was examined in lung and liver of A/HeJ mice by measuring BP-induced unscheduled DNA synthesis (UDS). BP treatment did not induce UDS in lung whereas UDS was observed in liver after BP treatment. 4-Nitro-quinoline-oxide (4NQ)-induced UDS was also examined. In contrast to BP, 4NQ induced UDS in lung but not in liver. Both BP and 4NQ induce neoplasia in lung of mice but not in liver. The observed *in vivo* disappearance of BP metabolite-DNA adducts in lung of these mice might be due to cell turnover.

   c. The formation of BP metabolite-DNA adducts in lung, liver and forestomach of control and BHA-treated (5 mg/g diet) female A/HeJ mice was examined as a function of BP dose (p.o.) ranging from 2 to 1350 μmol/kg. In untreated animals the dose-response curves for the adducts were sigmoidal in each tissue. In forestomach the dose-response curve for BPDE-DNA adduct levels in BHA-treated mice was parallel to the curve for control animals and thus, the inhibition (45%) of adduct formation was independent of BP dose. In contrast, BHA treatment diminished the curvilinear nature of the dose-response curves for BPDE adducts in lung and liver. The dose-response curves in lung and liver of treated animals were approximately linear. The inhibition in lung (70%) and liver (80%) was highest at a BP dose of 300 μmol/kg. As BP dose approached zero, the inhibition of BPDE-DNA adduct formation decreased with BP dose and approached values of 40% (lung) and 55% (liver). These results suggest that BHA treatment will also inhibit the neoplastic effects of BP at environmentally relevant doses of BP.

B. Cell Pharmacology Group (Leader: Dr. J. R. Fouts)

This group investigates the localization of drug and pollutant metabolizing enzyme systems in tissues that serve as interfaces with the environment (e.g., lung, skin and gut). This research group is also investigating factors which affect chemical-metabolizing systems, the development of these systems in the perinatal period, and on species differences in these systems. Cell types are isolated from these organs and enriched by e utriation and centrifugation techniques. The contribution of the metabolic systems in individual cells to target organ and cell toxicity is evaluated. Assay systems are being developed so that both oxidation and conjugation pathways of chemical metabolism can be quantitated in single cells. Such procedures will

eventaully be extended to other systems, including cells in culture. The scientists in this section frequently collaborate with those in the Molecular and Comparative Pharmacology group.

Another focus of interest is intestinal function and toxicology at the cellular, subcellular and molecular levels. A better understanding of the basic biochemistry, physiology and pharmacology of the normal intestine should permit greater appreciation for the unique roles of this organ in absorption and metabolism. In addition, this better understanding of normal function may lead to better methods for the detection of dysfunction and toxicity.

Recent Accomplishments:

1. Dr. Fouts' laboratory:

   a. The initial characterization of drug metabolizing systems in isolated lung cells of rabbit and rat (both MFO and conjugating systems) has been completed. The effect of ß-naphthoflavone induction on selected systems in the rat lung has been characterized.

   b. The role of cytochrome b_5, P-450 reductase and form 2 of P-450 in the metabolism of 7-ethoxycoumarin (7-EC) and p-nitroanisole in type II vs. Clara cells of rabbit lung has been studied in collaboration with Dr. Philpot's lab (antibodies for reductases, P-450 types). Role of b_5 seems quite different in different cells.

   c. Study of the metabolism of prostaglandins and arachidonic acid in rabbit type II vs. Clara cells; co-oxygenation vs. P-450-dependent metabolism of benzo(a)pyrene in lung cells (especially of rat, control vs. induced) has been made in collaboration with Dr. Eling's group. Qualitative and quantitative differences in type II vs. Clara cell prostaglandin metabolism were seen; BP metabolism after induction differed more in type II than Clara cells; marked rat vs. rabbit differences in prostaglandin metabolism.

   d. Study of acetylaminofluorene and benzo(a)pyrene metabolite patterns in rabbit lung cells -- in collaboration with Drs. Bend and Philpot (HPLC work and antibodies). Differences in amount of specific metabolites are seen in Clara vs. type II cells. Effects of antibodies to specific P-450's are being studied to see if this can clarify the differences in metabolite patterns.

   e. Study of the metabolism of 7-EC, 7-ethoxyresorufin (7-ERF) and benzphetamine (BzPH) by single isolated rabbit lung cells using the microspectrophotometer continue. Methodological problems have been minimized (diffusion of fluorescent product from cell) by use of 7-ERF and BzPH as substrates. Individual cell variation in activity documented (up to 10x).

   f. We have compared different methods of digestion for isolating skin cells, and have settled on Pronase, metrizimide gradients and elutriation to obtain reasonably pure fractions of basal cells (>80%) and sebaceous cells (>70%) and 8 fractions of the more differentiated cells of the layers between the basal cells and the skin surface. Morphology (light and EM),

enzymology (MFO and conjugations) and response to β-naphthoflavone (BNF) have been studied. Sebaceous cells have been compared in rats and mice with Zymbal's gland cells before and after treatment of animals with BNF.

g. Sulfate and glucuronide conjugation of the product of 7-EC metabolism (umbelliferone = UMB) was studied in a population of mixed epidermal cells. The ratio of sulfate to glucuronide products depended on substrate concentration. The possible limits of cofactor concentration on metabolism of 7-EC to UMB and further metabolism of UMB to conjugates were studied. Effect of inducers (BNF) on the two steps of metabolism of 7-EC in cells was also shown -- changes in ratios of free-to-conjugated UMB are marked when control is compared with induced sample. Liver was compared with skin (in control, skin conjugates only 30% of available UMB, while liver conjugates over 90%).

h. Study of the thermolability of the skate liver P-450 reductase was finished in collaboration with Dr. Philpot's group.

2. Dr. Schiller's laboratory:

a. An animal model system for the examination of regulation of lipid assimilation (transport and metabolism) was developed. The starvation-like effect of TCDD which is supported by weight loss had lead other investigators to suggest that nutrient absorption was blocked in some way. This suggestion is supported by the appearance of abnormally large lipid droplets in the gut cells after a lipid meal. Our in vitro and in situ experiments indicate no decrease in nutrient uptake, i.e., glucose and amino acid, and in the case of lipid, no decrease in uptake and resynthesis. A time-course experiment in control and TCDD-treated rats proved that lipid was taken up by the treated rat intestine by its appearance in the serum, i.e., that the level of triglycerides was greater in the treated rats and that the length of time for clearance through the intestines was also greater in the treated animals.

b. Methodology was developed to isolate chylomicra from fat-laden cells and chylomicra and other lipoproteins from mesenteric lymph and from serum. In addition, the lipoprotein fractions were delipidated and the apolipoproteins obtained were examined via SDS-gel electrophoresis.

c. Double-label experiments revealed a pattern of net protein synthesis in tip and crypt cell subcellular fractions on SDS-PAGE. The heterogeneity of synthesis rates within the mitochondrial fraction is of particular interest since it suggests a differentiation process in mitochondrial structure and/or function concomitant with crypt cell maturation and migration to the villous tip region.

d. The α-GP shuttle dominates in colon but not in liver. A pI 7.0 isozyme of cytosolic α-GPDH is seen in the colon during development but is appearance is blocked by treatment with 1,2-dimethylhydrazine.

C. Receptor Pharmacology Group (Leader: Dr. G. W. Lucier)

The research of this group is concerned with various aspects of hepatotoxicity emphasizing the more subtle alterations in liver function following exposure to environmental agents. The major focus is to characterize the role of endocrine action in the regulation of hepatic function in control and pollutant-treated animals, including the role of hormone receptors and toxicant-receptor interactions. The presence of receptors indicates that the liver is a target organ for estrogens and the study of hepatic estrogen-receptor interactions and the consequences of this estrogen action is clearly of importance in determining the impact of estrogenically-active chemicals on liver function.

The goal of these studies is to investigate the relationship of hepatic estrogen action to various forms of organ-specific toxicity including cardiovascular disease, hypertension and hepatotoxicity.

Recent Accomplishments:

1. Dr. Lucier's laboratory:

   a. Studies using the rat as an experimental model have shown that the liver contains estrogen receptors possessing criteria usually assigned to receptor proteins, and these receptors are associated with specific biochemical responses. The levels of liver estrogen receptor play a role in estrogen-induced production of very low density lipoproteins.

   b. An in vivo - in vitro approach was used to evaluate estrogen-mediated changes in protein synthesis by two-dimensional gel electrophoresis of newly-synthesized proteins.

   c. Demonstrated multihormonal regulation of different classes of estrogen binding proteins in liver and characterized the importance of the endocrine environment, at critical developmental stages, in regulating responsiveness of the liver to estrogens and androgens. One class of hepatic estrogen binding proteins might actually include androgen receptor. Regulation of receptor synthesis in liver is under pituitary control and this regulatory mechanism is different than in the uterus.

   d. Sex-differentiation of hepatic metabolism (including some of the drug-metabolizing enzymes) appears to be imprinted at birth by neonatal hormones during a critical developmental stage. Partially characterized the nature of pituitary regulation of specific forms of hepatic cytochrome P-450 and elucidated some of the age-specific endocrine parameters that ultimately regulate the levels and types of key metabolic activation/deactivation enzyme systems in liver.

   e. Studies have shown that hepatic cytosol contains one or more proteins which specifically bind androgens and exhibit several properties very similar to those of steroid receptors in other tissues including nuclear translocation and binding to DNA. In addition, these results indicate that the presence of this androgen binding protein correlates well with the ability of androgens to alter hepatic xenobiotic metabolism.

2. Dr. Fowler's laboratory:

a. Discovery and partial characterization of the 11,500 and 63,000 dalton Pb-binding proteins including tissue specificity (brain and kidney), K_d (10^{-7} - 10^{-8} M), binding capacity (14 pmol/mg cytosolic protein, 421 pmol/g kidney). Studies carried out on sucrose density gradients showed that binding of ^{203}Pb to the 63,000 dalton component was decreased with an increase in salt concentration (0-0.6 M $NaNO_3$). Competition studies with divalent cations showed 40-50% displacement of ^{203}Pb, bound to cytosolic components by Cd^{2+} and Zn^{2+} but no displacement with ion-exchange chromatography, showed an absorbance peak at 280 nm indicating the presence of aromatic amino acids and a high absorbance band between 200-230 nm.

b. Characterization studies of the renal brush border Pb transport system were conducted using membrane vesicles in vitro: 1) Pb uptake into vesicles is not influenced by Ca or other cations, valinomycin or Ca inophores, but is stimulated approximately twofold when the 63,000 (but not the 11,500) Pb binding protein is incorporated within the vesicles. Uptake is also increased in the presence of permeable anions and basic amino acids. Cysteine markedly reduces Pb uptake; 2) Pb binding to membrane vesicles was evaluated by vesicular volume changes, temperature sensitivity, equilibrium binding analysis (K_d = 1.4 X 10^{-10} M, binding capacity 1.5 nmol/mg protein) and accelerated exchange diffusion (exchange of cold intravesicular Pb with ^{203}Pb).

c. Circular dichroism studies on the oyster cadmium-binding protein (CdBP) have shown that this protein has a markedly different structure from metallothionein (50% β-sheet, 40% random coil) versus >95% random coil for metallothionein. This protein has only 2 Cd binding sites versus 7 for metallothionein but an absorption peak at 259 nm indicating a Cd-S bond similar to metallothionein suggesting that the essential nature of the binding site is conserved between phyla.

D. Collaborative Efforts

As can be seen from the individual project descriptions, scientists in the Laboratory of Pharmacology are involved in many activities and collaborative research efforts with scientists here at NIEHS and elsewhere. Especially noteworthy are the interactions at our marine laboratories in Maine and Florida where a wide variety of interdisciplinary research is carried out with a large number of scientists in residence at these laboratories.

Examples of collaborative programs outside of NIEHS for each of the senior scientists are: Dr. Bend with Dr. Bengt Mannervik of the University of Stockholm, and Drs. Don Reed and Mike Meredith of Oregon State University; Dr. Fouts with Drs. Mike Boyd and Charles Statham, National Cancer Institute, Dr. Ping Pan, U. S. Department of the Interior, Dr. Leakey at the University of Dundee and also with Dr. Meredith and Reed, Oregon State University; Dr. Lucier with Dr. Kern of the University of Colorado Medical Center; Dr. Philpot with Dr. Eric Johnson of Scripps Clinic and Research Foundation, Dr. Paul Thomas of Hoffman-LaRoche and Dr. Boyd of NCI; Dr. Pritchard with Dr. David Miller, Mount Desert Island Biological Laboratory; Dr. Anderson with Dr. Bob Dedrick, Chemical Engineering Section, NIH and Dr. Jim Selkirk, Oak Ridge National Laboratory; Dr. Fowler with Dr. David Engel, NOAA,

Department of Commerce, Dr. Kathryn Mahaffey of the Food and Drug Administration and Dr. Ian Armitage, Yale University; and Dr. Schiller with Dr. Boris Yagen of Hebrew University, Israel.

The collaborative efforts are cited only to show the extensive interactions of this Laboratory with groups outside NIEHS. In addition to these contacts, those with faculty and researchers in the Triangle area are too numerous to document, but add strength to our activities, peer reviews (in terms of seminars, discussions, exchange of students), and opportunities for advice, new techniques, and short courses not only for our staff but for members of the other institutions as well.

E. Personnel

New additions to the Laboratory of Pharmacology during FY'82 were Dr. Barbara Domin (Staff Fellow with Dr. Philpot), Dr. Tore Aune (International Research Fellow with Dr. Bend), Dr. Calliopi Mistry (Visiting Fellow with Dr. Lucier), Dr. Prakash Mistry (Visiting Fellow with Dr. Fowler), Ms. Diane Campen ("Q" appointment with Dr. Lucier), Mr. Dennis Chapman ("Q" appointment with Dr. Schiller), Ms. Jill Stowers ("Q" appointment with Dr. Anderson), and Mr. Robert Vanderslice ("Q" appointment with Dr. Philpot). Mrs. Geraldine Carver was also converted from a PPT position, GS-9 Biologist, to a PFT appointment, GS-9 Biologist, this year. Those leaving the laboratory were Dr. Larry Renfro (IPA with Dr. Pritchard), Ms. Jean Angelo (Technician with Dr. Pritchard) and Dr. Shelley Slaughter (Senior Staff Fellow with Dr. Lucier).

F. Other Activities

Dr. J. R. Bend: Adjunct Associate Professor, Department of Entomology, North Carolina State University, Raleigh; member, Executive Committee of Faculty of Toxicology, North Carolina State University; Adjunct Associate Professor, Curriculum in Toxicology, School of Medicine, University of North Carolina; member, Editorial Advisory Board for Drug Metabolism and Disposition and Environmental Health Perspectives: Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Chairman, Scientific Advisory Committee, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; Associate Editor, Biological Basis of Detoxication; Coordinator for "Special Topics in Toxicology" a graduate course given for the fourth consecutive year in Research Triangle Park for students at Duke, North Carolina State and University of North Carolina; served on graduate student committees at North Carolina State University and University of North Carolina; Invited participant at international symposium on the Use of Small Fish Species in Carcinogenesis Testing (Bethesda), at the Nobel Conference on Functions of Glutathione-Biochemical, Physiological and Toxicological (Sweden) and at the Fourth International Conference on Cytochrome P-450: Biochemistry, Biophysics and Environmental Implications (Finland); Presented seminars at Case Western Reserve University, to the Departments of Biological Chemistry and Pharmacology at the University of Michigan Medical School, Ann Arbor and at the Department of Biochemistry, University of Texas Health Science Center, Dallas; Also presented several graduate lectures in the Research Triangle area.

Dr. J. R. Fouts: Adjunct Professor of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill; Adjunct Professor of Toxicology, Department of

Entomology, School of Life Sciences, North Carolina State University at Raleigh; member, Toxicology Advisory Committee, Faculty of Toxicology, North Carolina State University; member, Epilepsy Advisory Committee, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda; counselor, American Society for Pharmacology and Experimental Therapeutics; editorial boards and editorial duties for many pharmacology journals. Lectures to graduate students at UNC - e.g.,- three lectures in Pharmacology 206, Biotransformation of xenobiotics and discussion panels. Served on graduate student committees - e.g.,- Lori Dostal, Department of Pharmacology, UNC and Jeffrey Boyd, Toxicology Curriculum, North Carolina State.

Dr. G. W. Lucier: Adjunct Associate Professor, Department of Biochemistry and Nutrition and Curriculum in Toxicology, University of North Carolina, Chapel Hill; Provisional member of Graduate School Faculty, University of North Carolina School of Medicine; Co-editor Environmental Health Perspectives; Editorial Boards of Peadiatric Pharmacology and Journal of Applied Biochemistry; Invited speaker to Toxicology Forum, in utero metabolic imprinting; International Agency for Research on Cancer, endocrine host factors affecting experimental carcinogenesis; Chinese Academy of Medical Sciences, Biochemical Toxicology; Ambulatory Pediatric Association, developmental biochemistry of liver; Western Ontario University, endocrine regulation of liver function; University of Florida, estrogen action in liver; Consultant to EPA on implementation of the Toxic Substances Act as it applies to children; Co-organizer of Research Triangle Park Receptor mechanisms discussion group.

Dr. R. M. Philpot: Adjunct Associate Professor, Department of Entomology, North Carolina State University, Raleigh; member, Toxicology Advisory Committee, North Carolina State University; Associate Managing Editor (U.S.A.), Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; member, Editorial Board, Molecular Pharmacology; Invited speaker at the symposium on Microsomes and Drug Oxidations, Tokyo, Japan, August, 1981.

Dr. M. W. Anderson: Adjunct, North Carolina State University, Biomathematics and Toxicology Department; member of the Committee on Pyrene and Selected Analogs, National Research Council, National Academy of Sciences; Seminar at North Carolina State University; Invited presentation at FASEB, 1982.

Dr. B. A. Fowler: Adjunct Associate Professor, Department of Pathology, University of North Carolina, Chapel Hill; member, Editorial Boards Chemico-Biological Interactions, Environmental Health Perspectives; Invited participant at the American Society for Pharmacology and Experimental Therapeutics (FASEB) Symposium on Metal Toxicity in the Kidney, University of Rochester International Conference on Developmental and Reproductive Toxicity of Metals (Session Co-chairman); Dahlem Conference on Changing Biogeochemical Cycles of Metals and Human Health (Rapporteur); Invited to present seminars at the Institute of Environmental Medicine, New York University, Duke University Marine Laboratory, Johns Hopkins University.

Dr. J. B. Pritchard: Adjunct Associate Professor, Department of Pharmacology, University of Florida School of Medicine, Gainesville.

Dr. C. M. Schiller: Adjunct Associate Professor, Department of Biochemistry and Nutrition, School of Medicine, University of North Carolina, Chapel Hill; member of the Faculty of the Graduate Curriculum in Toxicology, University of North Carolina, Chapel Hill; Liaison Member, U.S.-EPA Toxic Substances Subcommittee, Science Advisory Board, Washington, D.C.; member, Digestive Diseases Coordinating

Committee, Bethesda, MD; Alternate member, Nutrition Coordinating Committee, Bethesda, MD; Lecturer in graduate-level courses in Biochemical Toxicology at the University of North Carolina, Chapel Hill; Graduate advisor of students from the Department of Biochemistry and Nutrition and the Curriculum in Toxicology, University of North Carolina, Chapel Hill; Sponsor of NIH Postdoctoral Fellow in Toxicology Training Program; Invited lectures on "Mitochondrial Toxicity of Phthalate Esters," NTP Symposium, "Effects of Toxins on Gastrointestinal Function: Developing Systems, Cold Spring Harbor Symposium, and "Intestinal Gluconeogenesis and Metabolism," American Chemical Society Symposium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35005-03 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pharmacokinetic Considerations in the Formation and Repair of Carcinogen-DNA Adducts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marshall W. Anderson	Mathematician	LP	NIEHS
Other:	Dr. Mahmooda Kulkarni	Senior Staff Fellow	LP	NIEHS
	Ms. Jill Stowers	"Q" Appointment	LP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION
NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	1.7	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There is much evidence to suggest that the extent of carcinogen-induced promutagenic DNA damage and the capacity of cells to repair such damage represent critical events in the initiation of carcinogenesis. We are studying the in vivo formation and repair of benzo(a)pyrene metabolite-DNA adducts in target and non-target organs for benzo(a)pyrene-induced neoplasia. The effect of benzo(a)pyrene metabolite-DNA adducts on de novo synthesis is being investigated. We are concerned with the effect of dose of BP and inhibitors of benzo(a)pyrene-induced carcinogenesis on the amount and type of adducts formed. Emphasis is on studies which enhance our understanding of the relationship between metabolism of BP and the amount and types of DNA adducts formed in the various tissues.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1) To examine the in vivo formation and repair of benzo(a)pyrene (BP) metabolite-DNA adducts in various tissues and in various species.

2) To determine the rate-limiting step(s) in the in vivo formation of BP metabolite-DNA adducts.

3) To test the hypothesis that the extent of BP metabolite-DNA adduct formation and/or repair of such damage can explain the difference in organ and species susceptibility to BP-induced neoplasia.

4) To examine the effect of the initial amount of BP metabolite-DNA adduct formed on the enzymatic repair of the adducts in a target and non-target organ for BP-induced neoplasia as well as the effect of adduct formation on de novo DNA synthesis.

5) To examine the effect of inhibitors of BP-induced carcinogenesis on the formation of BP metabolite-DNA adducts under conditions known to result in inhibition of BP-induced neoplasia.

6) To examine the relationship between the metabolism of BP and the amount and types of BP metabolite-DNA adducts formed in the various tissues.

7) To investigate whether or not the amount of specific carcinogen-DNA adducts can be used as the effective dose in the low-dose risk estimation of chemical carcinogens.

METHODS EMPLOYED: 1) Animals were treated with various doese of ^{3}H-BP and then sacrificed at various time points. DNA was isolated from tissue by phenolic extraction plus hydroxyapatite chromatography. Isoalted DNA was enzymatically digested to individual nucleosides. A high pressure liquid chromatography (HPLC) procedure was developed to analyze for BP metabolite-deoxynucleoside adducts.

2) Carcinogen-induced unscheduled DNA synthesis (UDS) was examined by separating de novo DNA synthesis from repair synthesis (UDS) by alkaline CsCl gradients. BRDU pellets were implanted subcutaneously in animals to label the replicating DNA. ^{3}H-thymidine (i.p.) was used to label the DNA undergoing repair synthesis.

MAJOR FINDINGS AND PROPOSED COURSE: 1) BP metabolite-DNA adducts were observed in lung, liver, forestomach, brain, colon, kidney and muscle of A/HeJ mice after oral dose (6 mg/mouse) of ^{3}H-BP. The 7β,8α-dihydroxy-9α,10α-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEI)-deoxyguanosine was the predominant adduct observed. The 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEII)-deoxyguanosine adduct and an unidentified adduct, probably a BP-phenol-oxide-DNA adduct, were also detected in these tissues (8-15% and 10-20% of the BPDEI-deoxyguanosine adduct, respectively). Surprisingly, the specific activities (pmol/mg DNA) of the BP metabolite-DNA adducts did not vary more than 2-fold between these tissues. In contrast, there was more than a 20-fold variation between the tissues in binding of BP metabolites to protein. This data suggest that the metabolic capacity of a tissue might not be the rate-limiting step in the formation of BP metabolite-DNA adducts.

Adduct formation in these tissues will be examined at lower doses of BP as well as in other species. It is important to determine if, in general, BP metabolite-DNA adducts are being formed in most tissues after low dose exposure since BP as well as other polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants.

2) BP-induced DNA repair was examined in lung and liver of A/HeJ mice by measuring BP-induced unscheduled DNA synthesis (UDS). BP treatment did not induce UDS in lung whereas UDS was observed in liver after BP dose. 4-Nitro-quinoline-oxide (4NQ) induced UDS was also examined. In contrast to BP, 4NQ induced UDS in lung but not in liver. Both BP and 4NQ induce neoplasia in lung of mice but not in liver. The observed in vivo disappearance of BP metabolite-DNA adducts in lung of these mice is probably due to cell turnover. We will continue to examine BP-induced UDS as a function of dose and time in various tissues (see No. 1 of this section) of various species and strains of mice. We will also attempt to examine BP (4NQ)-induced UDS in both normal and replicating DNA.

3) The formation of benzo(a)pyrene (BP) metabolite-DNA adducts in lung, liver and forestomach of female A/HeJ mice was examined as a function of BP dose (p.o.) ranging from 2-to-1350 μmol/kg. The major identified adduct in each tissue at each dose was BPDEI-deoxyguanosine adducts. The BPDEII-deoxyguanosine adduct and a BP-phenol-oxide-DNA adduct were also observed. The dose-response curves for these adducts were sigmoidal in each tissue. The DNA binding expressed as percentage of exposure dose was largest at a BP dose of 300 μmol/kg. The dose-response relationships approached linearity at low doses. Thus, the results of these dose-response studies do not reveal the existence of any threshold dose below which binding of BP metabolites to DNA does not occur. Dose-response curves for binding of BP metabolites to protein were also investigated. The ratio of BPDE-DNA adduct levels to protein binding levels was non-linearly related to exposure dose. Thus, binding of BP metabolites to protein cannot be used as a gauge of BP metabolite-DNA binding. These and similar dose-response relationships for polycyclic aromatic hydrocarbons (PAH) metabolite-DNA adducts should be helpful in the low dose extrapolation problem for PAH carcinogenesis.

4) The phenolic antioxidant, BHA, has been shown to be a potent inhibitor of the neoplastic effects of BP in mouse lung and forestomach. We previously showed that BHA treatment of mice inhibited BP metabolite-DNA adduct formation in the lung to the same degree that BP-induced pulmonary adenoma formation was inhibited. In this study the formation of BP metabolite-DNA adducts in lung, liver and forestomach of control and BHA-treated (5 mg/g diet) female A/HeJ mice was examined as a function of BP dose (p.o.) ranging from 2-to-1350 μmol/kg. In untreated animals the dose-response curves for the adducts were sigmoidal in each tissue (see 3). In forestomach the dose-response curve for BPDE-DNA adducts levels in BHA-treated mice was parallel to the curve for control animals and thus, the inhibition (45%) of adduct formation was independent of BP dose. In contrast, BHA-treatment diminished the curvilinear nature of the dose-response curves for BPDE adducts in lung and liver. The dose-response curves in lung and liver of treated animals were approximately linear. The inhibition in lung (70%) and liver (80%) was highest at a BP dose of 300 μmol/kg. As BP dose approached zero, the inhibition of BPDE-DNA adduct formation decreased with BP dose and approached values of 40% (lung) and 55% (liver). These results suggest that BHA treatment will also inhibit the neoplastic effects of BP at environmentally relevant doses.

5) The effect of the aryl hydrocarbon hydroxylase (AHH) inducer, β-naphthoflavone (βNF), on BP-metabolite-DNA adduct formation has been examined under conditions known to result in inhibition of BP-induced neoplasia by βNF. Treatment of A/HeJ or ICR/Ha mice with βNF markedly decreased the amount of the BPDE-DNA adducts in lung, forestomach, and liver. There was approximately a 90% reduction in lung and forestomach and the adduct was not detectable in liver. The decrease in the formation of the BPDE-DNA adducts in the target tissue correlates with the inhibition of BP-induced neoplasia by βNF. The effects of two other AHH inducers, TCDD and Aroclor 1254, on in vivo BP-DNA adduct formation was examined. These inducers, like βNF, markedly decreased the formation of BPDE adducts. Thus, AHH inducers inhibit in vivo BPDE-DNA adduct formation in every tissue of every mice strain examined. We have initiated studies to examine the effect of AHH inducers on BP metabolite-DNA adduct formation in lung and liver of rabbit. The rabbit is a good model system for these studies as the cytochrome P-450 monooxygenase enzymes have been characterized in detail in lung and liver of the rabbit and the effects of AHH inducers on this enzyme system are documented. If BPDE-DNA formation is also inhibited in rabbit by treatment with AHH inducers, then the mechanism(s) by which AHH inducers inhibit the in vivo formation of BPDE-DNA adducts can be explored more readily in rabbits than in mice. These studies will be done in collaboration with Drs. J. R. Bend and R. M. Philpot.

6) Aspirin and indomethicin, inhibitors of prostaglandin synthetase (PG), had no effect on the in vivo formation of BP metabolite-DNA adducts in lung, liver and forestomach of A/HeJ mice after oral dose of ^{3}H-BP. Doses of aspirin and indomethicin were used which inhibited pulmonary PG. We also showed that treatment of A/HeJ mice with aspirin had no effect on BP-induced pulmonary adenomas. Thus, PG is probably not involved in the in vivo metabolism of BP. These studies were done in collaboration with Dr. Eling and Sivarajah of LPFT.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The extent of carcinogen-DNA adduct formation and the capacity of cells to repair such damage represent critical events in the initiation of carcinogenesis. The amount of adducts formed and/or their removal rates from target cells may be important factors in determining the susceptibility of organs or individuals to environmental carcinogens. For carcinogens such as BP, which require metabolic activation in order to exert their carcinogenicity, the amount of carcinogen metabolite-DNA adducts formed in a cell is a reflection of the quantitative balance between activation and detoxification pathways of the carcinogen. A detailed understanding of the correlation between mechanistic aspects of the metabolic processes for carcinogens and carcinogen metabolite-DNA binding profiles could result in our ability to predict and thus, protect certain individuals from carcinogen insult. Studies on inhibitors of carcinogenesis should provide some insight into this correlation. Moreover, understanding the mechanism of action of these anticarcinogenic agents would permit the rational design of more potent anticarcinogenic agents.

A problem of practical importance in environmental toxicology is to predict the potential chemical insult to man from high dose toxicology data in laboratory animals. Thus, both low dose and species-to-species extrapolations of toxicology data are involved in this prediction. Carcinogen-DNA adduct levels can usually be measured at much lower doses of the carcinogen than those used in bioassay studies. The potential use of the amount of carcinogen-DNA adducts formed in the

target cell as a measure of the effective dose of a carcinogen should help in the low dose and species-to-species extrapolation of tumorigenic data.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70132-03 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Regulation of Intestinal Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.0 or 3.0	1.5 or 2.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our research focuses on the development and use of animal model systems to study the regulation of gastrointestinal functions. Of particular concern are the regulation of intestinal absorption and metabolism of nutrients, and alteration of these occurring in response to oral exposure to biologically active environmental toxins. Current examples of these studies are: 1) the intestinal assimilation of nutrients in normal and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treated animals. The physiologic mechanisms of process regulation developed in these studies rely on in vitro and in vivo techniques; 2) the differentiation of intestinal cell and subcellular proteins; and 3) the role of NAD-linked dehydrogenases, for example, NAD-linked L-glycerol-3-phosphate dehydrogenase (NAD-2-GPDH), in the energy metabolism of the normal colon and 1,2-dimethylhydrazine-induced colon tumor.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Many of the techniques employed have been derived from those methods originally devised for other tissues, especially liver, and are described in the bibliographic references. Techniques that have been devised by us to monitor colon processes in Fischer rats include: 1) isolation and examination of colon cell mitochondria; 2) separation and purification of colon (or tumor or liver) cytosolic enzymes NAD-G3PDH, lactate dehydrogenase and L-malate dehydrogenase; and 3) analysis of isozymes of purified NAD-G3PDH, from liver, colon and colon tumor, by PAGE, IEF and SDS-gel electrophoresis. These techniques are described in detail in J. A. Alderman's Ph.D. dissertation and manuscripts (see bibliography).

Of special interest are the methodology that was necessary for us to establish in our laboratory to monitor intestinal nutrient assimilation. In vitro experiments with gut rings or gut sacs have been used by us for amino acid and monosaccharide or lipid uptake measurements, respectively. Specimens from whole animals were analyzed for metabolites (glucose, triglycerides, cholesterol), hormone (insulin), and enzyme (LP lipase) level changes. Lipid analyses were developed for evaluating serum, liver, mucosal cells, chylomicra and lymph. Chylomicra have been isolated from fat-laden individual intestinal cells and from the mesenteric lymph duct via cannulation. After purification, the chylomicra lipoproteins have been delipidated and examined by various gel electrophoresis techniques. The apoproteins are usually distinguished by molecular weight, amino acid composition and immunologic properties.

Analysis of gut cell proteins and apolipoproteins has necessitated the development of cell separation and protein purification techniques. Individual mucosal fat-laden cells are prepared by incubation with a collagenase solution, while crypt/tip isolation/separation is performed by a sequential chelation/vibrational technique. Markers for crypt/tip cells, for subcellular fractions (nuclei to post-microsomal supernate) and for submitochondrial fractions are used routinely. Cell proteins are labeled either in vivo or in vitro. In vitro double-labeled leucine experiments examine net rates of protein synthesis and in vivo experiments the onset of stimulated intestinal protein synthesis by a lipid meal. Isolated, purified and sometimes delipidated proteins are examined by PAGE, IEF, 2-D- and SDS-gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Lipid assimilation -- An animal model is being developed to monitor the regulation of lipid assimilation. Although previous morphologic and biochemical studies have established an intestinal cell response to a lipid meal as well as the subsequent events in the normal uptake, synthesis and transit of lipid through the lymph, the actual regulation of this essential response is not well defined. Our current research protocol is based on the observation in Fiseher rats by E.E. McConnel, that a prior single oral dose of TCDD alters lipid uptake as evidenced by large lipid droplets within the intestinal cell several hours after fat feeding. The end point of large lipid droplets formation during lipid assimilation was verified by transmission electron microscopy following a time course experiment. Indeed, the treated animal cells contain larger droplets than control animals, and these droplets are seen in the gut for a longer period of time.

Other changes we found in these animals resulting from TCDD treatment are a weight loss of 8% during the week following treatment despite access to food and apparently normal animal activity and movement. No animals died from the single oral dose of 99.5% TCDD (GC/mass spec.) at 53 μg/kg, based on neutron activation analysis of chlorine content of the corn oil solution, even during a 4-8 week waiting period. Several serum parameters were altered by the TCDD treatment as follows: protein increased to 117% of control; glucose decreased to 41% of control; triglycerides increased to 200% of control; free cholesterol increased to 182% of control and esterified cholesterol increased to 174% of control.

Several time-course studies indicated that the hypoglycemia observed in the treated rats is not related to hyperinsulinemia. In fact, the TCDD-treated rats exhibit a normal glucose tolerance test pattern with time, although the glucose levels are much lower throughout the 4-hour time-course than are those of the control rats. The serum insulin levels are not statistically different in the control and treated rats ($p > 0.05$). However, during the time-course, the treated animals demonstrated a more moderate insulin response to the glucose challenge. The interesting observation is that serum glucose levels are so low after TCDD treatment when the lipid levels are elevated. A disc gel (PAGE) profile indicated an aberrant pattern of α- and β-lipoproteins which suggested altered transport of lipids in these treated animals. The literature is incomplete as to whether TCDD alters lipid mobilization from the adipose tissue and also, into the liver. These are three areas of current and future work relating directly to lipid transport in the cell, lymph and serum.

Additional *in vitro* experiments showed that neither amino acid nor glucose transport into the intestinal tissue from the lumen was altered. Radiolabeled leucine or 3-O-methylglucose and gut rings were used to monitor the active transport via these nutrient carriers. An *in situ* experiment with radiolabeled palmitic acid demonstrated that lipid is readily taken up by the duodenum of the treated rat and is converted to the higher glycerides. The distribution of radiolabeled palmitic acid in the higher showed that each of the enzymes for intestinal triglyceride synthesis is present and active in a manner similar to the control animals, i.e., fatty acylthiokinase, monoglyceride acyltransferase and diglyceride acyltransferase. *In vitro* assay of the acyltransferases with radiolabeled palmitoyl-CoA and intestinal homogenates supported this observation.

The starvation-like effect of the TCDD which is supported by the weight loss has lead investigators to suggest that nutrient absorption is blocked in some way. This suggestion is supported by the large lipid droplets seen after a lipid meal. However, our *in vitro* and *in situ* experiments indicate no decrease in nutrient uptake and, in the case of lipid, no decrease in uptake and resynthesis. A relevant time-course experiment, in which corn oil (triglyceride) spiked with ^{14}C-triolein was fed to control and TCDD-treated rats, proved that lipid was taken up by the treated rat intestine by its appearance in the serum. Both serum radiolabel and triglyceride levels were measured over a 24-hour period. After background levels were subtracted, the time-course illustrated that the level of serum triglyceride was greater and the length of time for clearance through the intestine was greater in the treated animals than in the control animals. This difference was most noticeable 6 and 8 hours after fat feeding. The results of the time-course experiment may be explained by several experimentally testable hypotheses. 1) Lipid does not "clear" readily from the treated rat serum which leads to higher serum

levels. However, it is not known that triglycerides would back-up in the intestinal mucosa. 2) There is increased uptake of lipid by the intestinal epithelial cell and the normal processes lipid resynthesis and packaging cannot keep up which results in large lipid aggregates and finally increased levels in the serum. 3) The normal lipid resynthesis and packaging for transit is delayed or aberrant which results in large lipid droplets aggregating in the intestine and in higher triglyceride levels in the serum. Current experiments focus on uptake, packaging and clearance of lipid after a fat feeding. Once one or more of these hypotheses is/are implicated, the mechanism of this response and its alteration by TCDD can be examined more closely. Areas we are currently defining are the capacity of the treated liver to develop a fatty liver condition, a short time-course of lipid uptake and a dose-response effect with both morphologic and biochemical parameters as endpoints.

Net protein synthesis in isolated tip and crypt cells -- The intestinal mucosa presents a cell line that is differentiating as it migrates from the crypt to the tip regions of the villus. The relative rates of net protein synthesis in these two cell types are investigated by differential radiolabeling, ^{3}H-leucine -- crypt and ^{14}C-leucine -- tip, in vitro. Protein synthesis rates in rat duodenal enterocytes are roughly equivalent for both undifferentiated crypt cells and mature, fully differentiated tip cells. The radiolabeled proteins of the two cell types are mixed and separated by differential centrifugation into subcellular fractions (SCF), cell debris/nuclei, mitochondria, microsomes and supernate. When normalized to the ^{3}H/^{14}C ratio of the whole cell homogenate (WCH), the microsomes and mitochondria have ratios similar to the WCH, but the ratio of cell debris is higher, and that of the supernate lower. The SCF are then analyzed by SDS-polyacrylamide gel electrophoresis to determine any relationship between molecular weight (mw) and relative SCF net protein synthesis in the tip and crypt cells. Differences in protein synthesis rates do occur between cell types in some gross subcellular fractions with substantial heterogeneity existing within all SCF as determined by SDS-PAGE. Such heterogeneity has previously been shown for proteins specific to the functional requirements of the two cell types. The heterogeneity of synthesis rates within the mitochondrial fraction is of particular interest since this suggests a differentiation process in mitochondrial structure and/or function concomitant with crypt cell maturation and migration to the villous tip region. Further experiments addressing this question are being conducted.

Any direct correlation between leucine incorporation ratios and molecular weight in post-microsomal proteins is due to an inverse correlation between net protein synthesis and molecular weight in the tip cell. This is consistent with previously defined relationships between molecular weight and turnover rate. Interestingly, tip cell post-microsomal supernate is the only subcellular fraction from either cell type in which an inverse correlation is evident. It is possible that increased labeling intervals for duodenal enterocytes would result in similar correlations for other subcellular fractions. Alternatively, gut enterocyte protein turnover rates may not exhibit the same correlation with molecular weight as established in other tissues.

The gut enterocyte provides a unique model system for examining protein synthesis patterns in a differentiating epithelial tissue. The relative ease of obtaining mature vs. immature cell types makes this tissue particularly attractive for in vitro study. Although differential radiolabeling in vitro of villous tip and

crypt cells provides a useful technique for comparing relative net rates of synthesis for rapidly synthesized proteins and this procedure obviates the need for prolonged chase periods between labels typical of the in vivo double-label technique and therefore eliminates the problems associated with cell migration during the experiment, procedures for extending the in vitro labeling period for isolated enterocytes will be required before slowly synthesized proteins can be accurately examined.

Colon energy metabolism -- Studies of the α-glycerophosphate shuttle and of NAD-linked α-glycerophosphate dehydrogenase (NAD-α-GPDH) in the colon have revealed the following major observations: 1) the level and subcellular locations of the α-glycerophosphate and malate-aspartate shuttle enzymes were consistent with their proposed roles in reducing equivalents transport. K_m values of the shuttle enzymes were determined. Substrate shuttles were reconstructed using isolated mitochondria exhibiting satisfactory respiratory control and P:O ratios. The results suggest that while the malate-aspartate shuttle is the primary means of reducing equivalent transport in the liver, the α-glycerophosphate shuttle predominants in the colon. 2) The development of brush border hydrolases and enzymes of the malate-aspartate and α-glycerophosphate shuttles were evaluated in liver and colon of Fischer rats prenatally exposed to a 35 mg/kg oral dose of 1,2-dimethylhydrazine dihydrochloride. Results suggest that the specific activities of the developing malate-aspartate shuttle enzymes are more sensitive to 1,2-dimethylhydrazine treatment than are the activities of the brush border hydrolases or enzymes of the α-glycerophosphate. 3) Isozymes of partially-purified NAD-α-GPDH from rat liver, colon and 1,2-dimethylhydrazine-induced colon adenocarcinoma were examined by heat inactivation, disc polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing. Qualitative and/or quantitative differences in NAD-α-GPDH isozyme profiles among these three tissues were observed with each technique. This is the first time that distinctions between NAD-α-GPDH isozyme patterns of normal and well-differentiated tumor tissues have been described. 4) NAD-α-GPDH isozymes in liver and colon were examined during development of Fischer rats exposed in utero to a single high dose of 1,2-dimethylhydrazine. The NAD-α-GPDH was partially-purified and isozymes studied using heat inactivation, PAGE and isoelectric focusing. Several features of isozyme development were noted in both liver and colon. However, the only effect of 1,2-dimethylhydrazine treatment was to block the appearance of the pI 7.0 isozyme in adult colon. Characteristics of isozyme profiles from the youngest rats did not reappear in enzymes from colon tumor. 5) Rat liver NAD-α-GPDH and lactate dehydrogenase were almost completely purified and malate dehydrogenase partially-purified by affinity chromatography. 6) The apparent subunit molecular weight of all liver, colon and colon tumor NAD-α-GPDH isozymes distinguishable by PAGE was 35,000 ± 500 as determined by sodium dodecyl sulfate electrophoresis.

Proposed course -- We plan to concentrate our efforts in fiscal year 1983 on follow-up studies based on our animal model that we are developing for monitoring the regulation of lipid assimilation. Conceivably, at least three lines of investigation follow from our initial observations. First is the examination of the kinetics of the lipid uptake by the intestinal, epithelium, e.g., uptake of palmitic acid and 2-monopalmitic from bile salt micelles by everted gut sacs. Changes in kinetic uptake parameters of specific lipid substrates, under well-defined in vitro experimental conditions, may prove to be a valuable endpoint for correlating

physiologic response to treatment dose, for examining species/strain sensitivity and for evaluating toxin potency. Lipid uptake will be measured in Fischer rats as well as C57 and DBA mice with an everted sac preparation and with other in vitro organ culture techniques (D. Chapman). Second, regulation of packaging and transit of the absorbed lipid will be examined in the lipoprotein fractions, including chylomicra, from isolated intestinal cells, from mesentenic lymph and from serum. In addition to changes in chemical properties and composition, the control of the rates of synthesis and quality of the various chylomicra components will be analyzed with special emphasis on the apolipoproteins. The apoliproproteins are amenable to analysis by electrophoresis, chromatography and immunology (C. Shoaf). The third avenue of investigation is the regulation of the clearance of the lipid from the serum. This, of course, is a complex process involving, among others, the modulation of lipid flow from intestinal lymph, adipose tissue fatty acid mobilization and hepatic uptake of lipid. Initially, we will focus on the possible changes in the hormonal regulation of the serum lipoproteins and lipoprotein lipases that might explain the elevated lipid levels both 1 week after TCDD treatment and a few hours after fat-feeding (and 1 week after TCDD treatment) (R. Walden).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The role of intestinal metabolism to the overall homeostasis of the organism, nor the regulation of intestinal metabolism are well-defined. In general, intestinal epithelial metabolism may be expected to be even more complex than that of other tissues because of constant cell turnover with short half-life and additional function in the absorption of essential nutrients. Our studies of colon energy metabolism and intestinal cell protein synthesis are at the forefront of the biochemistry of intestinal metabolism and add to the basic knowledge and understanding of these processes.

Our observations in developing an animal model for examining lipid assimilation allow for further basic studies of this essential process. These studies should provide a deeper understanding of the kinetics of lipid uptake and the onset of intestinal apolipoprotein synthesis in response to lipid in the intestinal lumen. TCDD may prove useful as a probe to the understanding of the regulation of the complex process of lipid assimilation and the possible involvement of microsomal processes.

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Schiller, C. M. (ed.): Target Organ Toxicity: Intestines. Target Organ Series, Raven Press. (Invited).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70200 -08 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cellular Mechanisms of Metal Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D.H. Petering	NIH Senior Fellow	LP	NIEHS
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6.6	4.6	2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Animals exposed to different trace metals for prolonged periods of time show metal-specific biological response profile which specifically characterizes exposure to that metal. The objective of these studies is to assess and characterize response profiles based on a thorough understanding of subcellular mechanisms of metal toxicity and specifically to (1) define and correlate ultrastructural and biochemical responses in vivo which characterize exposure to toxic trace elements and (2) develop early, specific, and sensitive biochemical testing procedures that may be used to evaluate human populations exposed to environmentally important trace elements. Specific metals and areas of interest include the biochemical effects of cadmium and lead on cell membrane and mitochondrial structure and function. The relationships between intracellular molecular binding of lead and cadmium and toxic responses of organelle systems are also of intense concern. Cytosolic lead-binding components were found in target tissues for this element and were partially characterized by gel chromatography, electrophoresis and saturation analysis. The binding components appear to be a major initial intracellular compartment for lead entering the cells of target organs. Zinc deficiency enhances cadmium toxicity and increases the amount of non-metallothionein cadmium in rats following cadmium exposure.

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy, x-ray microanalysis; biochemical assays of mitochondrial respiration; and lysosomal, microsomal, cytosolic, and mitochondrial enzymes. Sephadex and DEAE-chromatography, electrophoresis, isoelectric focusing and amino acid analysis of metal binding proteins from mammals and marine shellfish. Tissue analysis for metals by atomic absorption spectroscopy and gamma emission spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Subcellular binding of ^{203}Pb in rat kidneys - Detection and partial characterization of cytosolic lead-binding components -- The subcellular compartmentation of a tracer dose of ^{203}Pb was studied in kidneys of control rats and rats given a single intraperitoneal injection of Pb acetate (15 mg Pb/rat) 6 days earlier. Intranuclear and cytoplasmic inclusion bodies were observed by electron microscopy in proximal tubule cells of Pb-treated rats. Kidneys of rats injected with ^{203}Pb acetate (30 µg Pb/rat) 24 hr prior to sacrifice were fractionated into crude and purified nuclear inclusion body, mitochondrial and cytosolic components. ^{203}Pb activity was two-times higher in kidney homogenates of controls compared with Pb-treated rats. This ratio was also observed in the mitochondrial and crude nuclear fractions whereas cytosolic ^{203}Pb activity from control rats was five-times higher than Pb-treated rats. Purified nuclear and inclusion body fractions from Pb-pretreated rats contained 10- and 25-times higher levels of ^{203}Pb radioactivity, respectively, compared with controls. Gel chromatography of the cytosolic fraction from control rats 2 hr after injection of ^{203}Pb revealed the presence of two major lead-binding components with molecular weights of about 11,500 and 63,000 daltons. These components were not observed in the cytosolic fraction of Pb-pretreated rats. The 11,500 dalton peak did not incorporate ^{14}C-leucine, and concomitant administration of cycloheximide with ^{203}Pb did not inhibit incorporation of ^{203}Pb activity, suggesting prior formation of the component. These studies indicate that formation of Pb intranuclear inclusion bodies alters the intracellular compartmentation of Pb and that previously undescribed cytosolic Pb-binding components exist in kidneys of non-Pb-treated rats. These cytosolic binding components were also observed in the cytosol of brain, a second target organ for lead toxicity, but not in liver or lung, suggesting a target tissue-specific localization for these lead-binding macromolecules.

Biochemical characterization studies using Sephadex G-200 column chromatography, SDS-polyacrylamide gel electrophoresis (SDS-PAGE)/autoradiography, sucrose density gradient analysis (SDGA) and saturation analysis following *in vitro* incubation of control rat kidney cytosol with various concentrations of ^{203}Pb for 60 min at 4°C prior to analysis. Sephadex G-200 column chromatography isolation of the 63,000 dalton ^{203}Pb binding component followed by simultaneous SDS-PAGE/autoradiography of this peak and the intact cytosol fraction confirmed that the 63,000 dalton component represented the major soluble ^{203}Pb binding constituent. SDGA of the cytosol disclosed a sirgle 4.6S peak which also contained most of the ^{203}Pb binding component in this fraction. Preliminary saturation analysis of the ^{203}Pb binding component showed a dissociation constant (K_d) of 10^{-8} M. These studies indicate that the 63,000 dalton peak is the primary cytosolic ligand for Pb in rat kidney due, in part, to its high affinity for this element. Such data are essential to understanding the potential role of this ligand in mediating the cellular uptake of Pb and formation of cytoplasmic/nuclear inclusions in target tissues for Pb toxicity.

The mechanism by which lead is transported into renal proximal tubule cells was studied using isolated plasma membrane vesicles from rat kidney. Vesicles prepared according to the technique of Beck and Sacktor were incubated with ^{203}Pb with carrier Pb acetate under a variety of conditions. At timed intervals (0-90 min), the reactions was stopped by addition of cold buffer and rapid Millipore filtration. Washing the vesicles with buffer containing 1 mM Pb acetate or 1 mM $CaCl_2$ showed that Pb was more effective in displacing nonspecifically-bound label and hence, all experiments were performed with 1 mM Pb acetate in the stop bath. Lead uptake was found to be time-dependent and appeared to saturate at 100 μM Pb in the incubation mixture. To differentiate between Pb uptake and binding, Pb uptake was studied in vesicles whose volume was osmotically altered by sucrose addition to the incubation media. It was found that Pb concentration in the vesicles varied directly with vesicle volume indicating uptake. Vesicle incubation in either a NaCl or KCl gradient (extravesicular > intravesicular concentration) increased the initial rate of Pb uptake by approximately 50% but not the final equilibrium value. Incubation of vesicles at 0°C did not alter Pb uptake; however, incubation with either 5 mM EDTA or EGTA reduced Pb uptake to approximately 60% of control values. The data indicate that Pb is extensively bound to the brush-border membrane under in vitro conditions but also demonstrate that accumulation occurs within the membrane vesicles and that the rate of this process can be influenced by extravesicular ion composition. Preliminary studies incorporating the 63,000 dalton ^{203}Pb binding cytosolic component showed an approximately 2-fold increase in vesicle transport of ^{203}Pb.

The effects of zinc deficiency (Zn) on cadmium (Cd) induced changes of tissue Zn and copper (Cu) were studied in young male rats exposed to 100 ppm Cd in drinking water for 30 days and then placed on Cd-free, Zn^- or normal (Zn^+) diets for 1 to 2 weeks. Total Cd decreased 15% in the liver and increased 10 to 20% in the kidneys of Cd/Zn^- rats relative to Zn^+ animals. Although most of the hepatic Cd was bound to metallothionein (CdMt) in the cytosol, the amount of Cd per mg protein was highest in mitochondrial-lysosomal and microsomal fractions. The Cd liberated from these fractions by sonication was bound to Mt. Zn^- treatment did not affect the binding of Cd to cytosolic Mt but increased non-Mt binding organelle compartments. Elevated hepatic and renal Zn levels in Cd-Zn animals returned to untreated, control levels in Cd-Zn rats. Plasma Cu levels decreased progressively in response to Zn^-, Cd, and Cd-Zn treatments. Kidney and heart Cu were elevated 150 to 230% in Cd-Zn^+ animals. The Cd-Zn^- treatment reversed these effects. The Zn-dependent enzyme, δ-amino levulinic acid dehydratase in erythrocytes was unchanged in Cd-Zn^+ rats but was depressed 50% in Cd-Zn rats. These data indicate that Zn deficiency alters tissue responses to Cd exposure in vivo with respect to Zn and Cu metabolism and at least one important Zn-dependent enzyme. Because of the sequential protocol employed, these effects do not appear to result from changes in absorption of metals in the gut.

In addition, further physical characterization studies were conducted on the oyster cadmium-binding previously discovered in this laboratory with Dr. Colin Chignell of the Laboratory of Environmental Biophysics. Preliminary cadmium saturation studies showed that although CdBP normally contains only one g atom of Cd/mol of protein, the addition of excess $CdCl_2$ to the supernatant during isolation appears to increase the amount of bound Cd 2-fold. At pH 7.35, CdBP exhibits a positive circular dichroic band at 259 nm which is abolished at pH 3.0. This band has been

attributed in methallothinein to an extrinsic Cotton effect originating from the Cd-sulfur bond. The circular dichroism of CdBP in the 200-220 nm range indicates that 50% of the protein exists in a β-pleated sheet conformation while the remainder (~40%) is random. These studies suggest that, like metallothionein, the binding of Cd to CdBP involves Cd-sulfur bonds. The lower Cd-binding capacity of the protein is consistent with the fact that it contains less cysteine. The lower cysteine content and the more ordered structure of CdBP support the view that this protein is not a true metallothionein.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal metabolism toxicity following prolonged exposure by combined ultrastructural and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations. In particular, metal-specific porphyrinurias and proteinurias should indicate the early development of metal toxicity. Such indicators would have potential applicability to populations living near fossil fuel power plants.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 71000-03 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Estrogen and Androgen Action in Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Lucier	Research Chemist	LP	NIEHS
Other:	O. McDaniel	Bio. Lab. Tech.	LP	NIEHS
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	R. Rumbaugh	Staff Fellow	LP	NIEHS
	S. Slaughter	Staff Fellow	LP	NIEHS
	T. Sloop	Bio. Lab. Tech.	LP	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LP	NIEHS
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	D. Campen	Graduate Student	LP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range plan of this project to study and understand changes in hepatic function following exposure to environmental agents emphasizing effects of hormonally active chemicals. These studies are defining the liver as a target organ for estrogens and androgens by characterizing cytosolic and nuclear steroid-binding proteins and correlating the presence of receptors with steroid-mediated induction or repression of protein synthesis. Some functional biochemical components of estrogen and androgen action in adult liver appear to be imprinted during a critical neonatal period by endogenous hormones. The imprinting of sex-dependent hepatic receptor synthesis is also evaluated in these studies. The pituitary-hypothalamic-hepatic-axis appears to regulate the ontogeny of hepatic metabolic steroid-binding proteins and the mechanisms involved are investigated in whole animal and culture systems. Environmental estrogens such as zearalenol mycotoxins, DES, and methoxychlor are assessed for estrogenic potency in liver.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES AND METHODS EMPLOYED: I. Investigate the nature of cytoplasmic estrogen and androgen binding proteins in rat liver.

A. Specific receptors. Separation of specific receptors from other non-specific estrogen binding proteins is accomplished by ammonium sulfate precipitation. Specific binding characteristics of androgens and estrogens are assessed by several criteria:

Characteristic	Technique
Finite binding capacity	Scatchard analysis
High binding affinity	Calculation of equilibrium binding constants from Scatchard plots
Distinctive sedimentation coefficients	Sucrose-gradient analysis
High binding specificity	In vitro competitive binding

B. Investigate the nature of higher capacity lower affinity (HCLA) binding sites. Unlike classical estrogen target tissues, the liver contains high levels of high capacity estrogen binding proteins. Binding characteristics of HCLA sites in male or female liver are determined by sucrose gradient analysis, gel filtration and polyacrylamide gel electrophoresis.

II. Investigate whether or not quantity and/or function of hepatic estrogen and androgen receptors or HCLA sites undergo sex differentiation. Studies outlined in Parts I and II suggest that levels of HCLA sites may be imprinted at birth through the pituitary-hypothalamic axis by testicular androgens. To further investigate these findings, rats are gonadectomized immediately after birth and given replacement hormone therapy (hormones, ectopic pituitaries, etc.) at different developmental stages. In addition, endogenous hormones and estrogenically active xenobiotics are administered to intact neonates to determine sensitivity of hepatic sex differentiation of binding proteins to changes in hormonal milieu during a critical period of early development.

III. Study the nature and sex differentiation of nuclear estrogen and androgen binding proteins in rat liver. These studies investigate: 1) nuclear translocation of specific ligand-receptor complexes into male and female rat liver nuclei and 2) differences in the nuclear translocation process between liver and classical estrogen and androgen target tissues. Nuclear binding is investigated in vivo and in vitro (tissue minces, cell free systems, isolated hepatocytes and isolated perfused liver). Binding is analyzed by density gradient centrifugation and exchange assays.

IV. Elucidate the role of HCLA sites in the nuclear translocation process. These studies compare the rate of uptake and nuclear retention of estradiol receptor complexes in relation to the quality and quantity of HCLA sites. Specific profiles of HCLA sites are produced by partial purification and endocrine manipulations of the neonatal adult animal. Techniques are the same as outlined in Part III.

V. Characterize the role that estrogen and androgen receptors and HCLA sites play in biological, biochemical and physiological response of the liver to estrogens and androgens (including estrogenically active xenobiotics). Indicators of hepatic responsiveness are serum triglycerides, production of specific lipoproteins, specific components of cytochrome P-450, analysis of protein synthesis in isolated hepatocytes by 2-dimensional gel electrophoresis, and analysis of protein synthesis using in vitro translation assays.

VI. Purify estrogen and androgen responsive proteins for the purpose of developing probes for steroid hormone action in liver.

MAJOR FINDINGS AND PROPOSED COURSE: Liver is considered a target organ for estrogens and contains estrogen receptor (ER). A second class of estrogen binding proteins, which exhibit higher capacity, lower affinity (HCLA) binding than ER, has also been detected in liver. HCLA sites bind steroidal androgens and estrogen but not nonsteroidal estrogens or other steroids. HCLA sites undergo sex differentiation such that adult male levels are 10-fold higher than adult female levels. Normal male levels of HCLA require neonatal exposure to androgens during a brief critical period of development. The levels of HCLA sites are affected by androgens and estrogens in an age-dependent way. Administration of testosterone (T) to castrated males during the critical period (days 6-16) imprints for sex differentiation of HCLA sites, whereas T administration at other times does not. Treatment with DES or zearalenol is incapable of restoring development of normal levels at any age. However, DES given to intact males produces age-specific effects. When treated with DES on days 2 and 6, intact males fail to develop normal levels of HCLA sites. DES administration on days 9 and 13 has no effects. The level of HCLA sites in adult castrated males is feminized by DES and estradiol but not zearalenol. These studies demonstrate an age-specific hormonal regulation of hepatic HCLA sites, which can be disrupted by agents that alter the hormone environment at various stages of development.

Further studies were undertaken to determine if HCLA sites might modulate receptor-mediated events. Sephadex column chromatography indicates that the HCLA sites are comprised of several proteins. A moderate affinity site ($K_d=4x10^{-7}$) is detected in male liver only, whereas lower affinity, non-saturable binding sites are present in both sexes, but are quantitatively greater in males. These sex differences are a consequence of imprinting by testicular androgen during a critical neonatal period. Neonatal castration feminizes levels of HCLA sites. In vitro nuclear uptake of cytosolic receptor-ligand complexes is more efficient in females as compared to intact males. Moreover, nuclear uptake of receptor-ligand complexes in neonatally castrated males is similar to that seen in the adult female. Increased levels of serum triglycerides associated with VLDL have been measured as an indicator of hepatic responses to estrogen (E). Female rats appear to be more sensitive to E exposure than males. Interestingly, Scatchard analysis of nuclear binding sites in E-treated revealed an inverse correlation between amount of nuclear sites and the sex-mediated differences in E responsiveness. Following neonatal castration, a marked increase in E sensitivity is observed in adult males, which can be reversed by neonatal testosterone treatment. These studies have demonstrated that the existence of HCLA sites influence the response of the liver to E.

In an attempt to more precisely characterize E influences on hepatic function, we have employed 2-D gel electrophoresis to analyze the protein synthetic profile of liver cells. These studies were conducted to determine whether qualitative and/or quantitative changes in the protein composition of isolated hepatocytes occurs following estradiol (E_2) pretreatment in vivo. Sections (8 mm) of silastic tubing containing E_2 or vehicle (cholesterol) were implanted sc into adult male, female or ovariectomized (ovx) female rats. Following 2 weeks of hormone administration, hepatocytes were isolated by collagenase perfusion. Isolated cells (2 ml at 3 X 10^6 cells/ml) were incubated in media, deficient in cystine (cys) and methionine (met), at 37°C for 3 hours with aliquots (250 µl) of [^{35}S] cys and met added at 0, 1 and 2 hours. Following incubation, cells were solubilized in 8 M urea and samples analyzed by isoelectric focusing (pI range 5-7) in the first dimension and SDS-PAGE in the second. The 2-D profile of proteins synthesized by hepatocytes isolated from control and E_2-treated animals reflects the translation of existing mRNA, produced in vivo. Comparison of the 2-D gel autoradiographs reveals several interesting sex differences. In particular, a major male liver protein with a pI of approximately 6.2 and a MW in the range of 20-30K has been identified which is virtually non-existent in female liver. Furthermore, the synthesis of this protein is completely suppressed when animals were pretreated with E_2. At least 3 proteins have been shown to be E-dependent in the female liver. Whereas ovx results in diminished synthesis of the three proteins, hormone treatment of ovx females partially restores synthetic capacity to that seen in the intact rat. These E-dependent proteins have an MW of approximately 60-70K with pI's ranging from 5.8 to 6.8. Additional studies have compared protein synthetic profiles of rats treated with steroidal (E_2) or non-steroidal (DES) estrogens. Using a combined in vivo - in vitro approach, we have demonstrated pronounced and selective alterations in hepatic protein synthesis following estrogen treatment.

As described above, we have demonstrated two classes of cytosolic estrogen binding proteins in liver of male and female rats. One, which sediments at 8S on sucrose gradients, has low capacity, high affinity sites and is similar to estrogen receptors (ER) in reproductive tract. The other has higher capacity, lower affinity sites and sediments at 4S. HCLA sites modulate action of estrogen in liver by regulating availability of ligand to ER. Both classes of sites have been shown to be under hypophyseal control. Animals were hypophysectomized (Hx) at day 23, 40, or 60 of age and implanted with EP from age- and sex-matched control animals between day 70 and 80. Controls received sham operations. Hx eliminated the sex difference in HCLA sites independent of age of Hx. The EP in Hx males or females produced female levels of HCLA sites regardless of age of Hx. Hx, at any age, eliminated the 8S ER in both sexes. The EP had no effect on the ER in prepubertally Hx animals. However, in animals Hx postpubertally, the EP partially restored the hepatic ER. These results suggest that the pituitary can secrete a factor(s), not normally present in the intact male, which can feminize the levels of hepatic estrogen binding proteins. There is also an age-dependent ability of the EP to secrete one or more factors that control the development of liver ER.

HCLA sites bind both androgens and estrogens with moderate affinity. Furthermore, these sites were shown to be regulated by a complex interaction of gonadal hormones. At least one of the HCLA sites may function as an androgen receptor whose ligands control its level with androgen stimulating and estrogen repressing receptor synthesis (Roy, Biochim. Biophys. Acta 354:213, 1974). Many hepatic microsomal

drug- and steroid-metabolizing enzymes are steroid responsive in a manner analagous to the hepatic androgen binding protein (ABP). The present study was designed to determine the functional role of hepatic ABP and whether its presence correlates with the presence of androgen inducible microsomal drug metabolism. Analysis of hepatic ABP was performed using ^{3}H-5α-dihydrotestosterone (DHT) as the ligand in intact and Hx rats of both sexes. Hepatic cytosol from male rats prelabeled with DHT showed a time-dependent translocation of radioactivity to purified hepatic nuclei in a cell-free system. Hepatic ABP of male rats bound to columns of denatured DNA cellulose and was eluted by a salt gradient in three peaks. This DNA binding ability was absent in livers of female rats and reduced in livers of Hx rats. Hepatic ABP is absent in immature male and mature female rats. In Hx rats of either sex, the levels of hepatic ABP are greater than those of female rats but less than normal males. The presence of hepatic ABP correlated with the ability of androgen to induce hepatic microsomal ethylmorphine N-demethylase (EM). EM activity was high in males, low in females, and low in hypox animals. Treatment of Hx animals with testosterone did not induce either hepatic ABP or EM demethylase. Treatment of male rats with estradiol feminized levels of both hepatic ABP and EM. The results suggest that the presence of hepatic ABP coincides developmentally with androgen induction of microsomal mixed function oxidase. The presence of an intact pituitary is required for expression of normal male levels of both hepatic ABP as well as EM demethylase. These observations are consistent with the concept that androgen action in the liver occurs through a receptor mechanism analagous to other steroid-responsive tissues.

The hepatic cytochrome P-450-dependent monooxygenase system is responsible for the biotransformation of a number of endogenous and exogenous substrates. Some of the oxidative metabolic enzyme activities exhibit sex differences which appear at puberty in the rat. The following is a study of the role of the pituitary in this sexual dimorphism. Hypophysectomy of postpubertal rats (60 days of age) abolishes sex differences in microsomal P-450 content, ethylmorphine demethylase (ED) and benzo(a)pyrene hydroxylase (BP) activities; P-450 content increases in both male and female rats, whereas microsomal ED and BP activities decrease in male animals and increase in female animals. NADPH-cytochrome c reductase, which exhibits no sex difference, is reduced by hypophysectomy. Growth hormone (GH) administration (5 mg/kg/day for 3 consecutive days) to Hx male rats partially reversed the effects of hypophysectomy (i.e. ED and BP activities increased). In Hx female rats, GH had a feminizing effect (decreased P-450 content, BP and ED activities). Transplantation of age- and sex-matched pituitary glands under the renal capsule of Hx recipient rats resulted in feminization of enzyme activities in male as well as female rats. Hypophysectomy results in a shift in the ratio of slow turnover to fast turnover P-450 components and changes in the microsomal protein profile as analyzed on SDS-polyacrylamide gels. The profile of Hx males showed an increase of several protein bands; most notably bands at M_r 49K, 54K, and 62K. The profile of Hx female rats was significantly different from that of sham-operated female animals and indistinguishable from Hx male rats. The correlation between increased P-450 content, shifts in P-450 turnover profiles, and microsomal protein electrophoretic profiles suggest that hypophysectomy results in a change in the relative amounts of different forms of P-450. Partial purification on DEAE-cellulose of solubilized microsomal preparations reveal that the major P-450 peak in Hx preparations had several proteins which were absent or significantly reduced in sham-operated animals. GH (or ectopic pituitary transplant) partially abolished the increase in

protein bands. These studies provide further evidence that the pituitary secretes a factor(s) which feminize certain hepatic enzymes and that sex differences in hepatic biochemistry are a consequence of these factors.

PROPOSED COURSE: Further studies will examine the biological/biochemical response of the liver following administration of androgens, endogenous estrogens or estrogenically active environmental agents. These studies will utilize in vivo systems as well as primary culture and isolated hepatocytes. Indicators studies will include molecular aspects of the estrogen-induced production of renin substrate, triglycerides, VLDL (very low density lipoproteins), protein synthesis as analyzed by 2-dimensional gel electrophoresis, specific components of cytochrome P-450 using reconstituted systems and in vitro translation of steroid-sensitive proteins using mRNA from treated animals. Sensitive markers will be purified for the purpose of developing RIA procedures for detecting steroid-mediated events in liver. Estrogen action in relation to cell type and location will be studied. The quality and quantity of hepatic steroid-binding proteins will be manipulated by surgical procedures such as hypophysectomy and castration and by altering the normal neonatal imprinting of specific-binding proteins. These types of studies should provide further insight into the role that estrogen- and androgen-binding proteins play in steroid-induced hepatotoxicity. The age-dependent response of the liver will also be investigated. The long-term goal is to correlate receptor level and type with responses of the liver and other systems to endogenous estrogens and to determine if estrogenically active chemicals produce the same type of response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During recent years it is becoming increasingly evident that the liver may be a toxicologically importat target organ for estrogens. For example, there are many adverse side effects of oral contraceptives that could be related to estrogen action in the liver. Some of these side effects are increased incidence of 1) thrombosis, 2) heart attacks, 3) jaundice, 4) gall stones, 5) hypertension, and 6) benign hepatomas. The occurrence of thrombosis may be related to increased synthesis of plasma clotting factors; cardiovascular disease could be enhanced by increased hepatic synthesis of plasma triglycerides and certain lipoproteins; estrogen alters the hepatic transport of bile acids and bilirubin and therefore might be a cause of jaundice; gall stones could result from the finding that estrogens increase cholesterol concentrations in bile; estrogen-induced hypertension could be caused by increased hepatic synthesis of renin substrate. Many environmental agents possess direct estrogenic activity, i.e., they bind to estrogen receptors. Therefore, a critical need exists to determine if the cellular machinery required for estrogen action is present in the liver and to ascertain whether or not the biological and/or toxic responses to estrogens in the liver is associated with specific forms of estrogen-binding proteins. Furthermore, we need to ascertain if the molecular interactions of the liver to steroidal and non-steroidal estrogens are the same or different. We have characterized specific hepatic cytosolic receptors for estrogens and demonstrated nuclear translocation of estrogen receptor complex in a cell-free system using the rat as an experimental animal. Additionally, our studies are investigating sex differences in estrogen action and the similarities and differences of estrogen action in the liver compared to other target tissues such as the uterus. In addition to directly affecting hepatic function, hormones might also regulate hepatic responsiveness to other xenobiotics. Because some aspects of liver biochemistry and physiology undergo postpubertal sex-differentiation,

it might be expected that a corresponding differentiation could occur in the interactions of chemicals with liver cell components. Sex-differentiation of hepatic metabolism is under pituitary-hypothalamic control and (including the drug-metabolizing enzymes) appears to be imprinted at birth by neonatal hormones during a narrow critical developmental stage. Therefore, alterations in the hormonal milieu during this critical period could irreversibly change the susceptibility of the liver to hepatotoxins. Previous studies have shown that neonatal estrogens can increase the incidence of chemically-induced hepatocarcinoma (Weisburger et al., Endocrinology, 82: 685, 1968). Since environmental chemicals may directly (receptor interactions) or indirectly (modification of metabolism and/or clearance of endogenous chemicals) elicit changes in hormone action, it becomes important to investigate the role of hormonally active chemicals in the generation of groups at risk to various forms of organ-specific toxicity.

PUBLICATIONS

Lamartiniere, C. A., Illsley, N. P., Kita, E. and Lucier, G. W.: Neonatal treatment with o,p-DDT or methoxychlor alters the imprinting of hepatic monamine oxidase. Biochem. Pharmacol. (In Press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80001-10 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Microsomal Mixed-Function Oxidase Systems: Specificity and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard M. Philpot Research Chemist LP NIEHS

COOPERATING UNITS (if any)
Laboratory of Molecular Genetics; Clinical Pharmacology Branch, National Cancer Institute; Dept. of Biochemistry, Scripps Clinic and Research Foundation; Dept. of Anatomy, School of Veterinary Medicine, University of California, Davis, CA

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Laboratory of Pharmacology

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4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO) from various sources. Present work involves the purification of cytochrome P-450, cytochrome b_5, NADPH-cytochrome P-450 reductase and NADH-cytochrome c reductase from rabbit pulmonary and hepatic microsomal fractions. Components of the MFO are being examined by UV-vis spectroscopy, electron paramagnetic resonance spectroscopy, SDS-gel electrophoresis, and by their activities in reconstituted systems. Structural and immunochemical properties of the enzymes are also being investigated. The long-range objectives of this work are to determine the influence of: 1) multiple forms of the enzymic components of the MFO systems, 2) endogenous compounds, and 3) exogenous compounds (substrates, inducers and inhibitors) on the substrate specificities of MFO systems from different tissues and species.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cytochrome P-450 is purified from rabbit pulmonary and hepatic microsomes by procedures developed in this laboratory. The purification of other enzymes is accomplished by published methods which are modified as required.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The synthesis of cytochrome P-450, form 5, is induced in liver following treatment of rabbits with phenobarbital -- The two major isozymes of cytochrome P-450 in rabbit lung are forms 2 ($P\text{-}450_I$) and 5 ($P\text{-}450_{II}$). Both of these enzymes are found in rabbit liver but only in very low concentrations. It has been known for some time that the synthesis of form 2 is induced in the liver following treatment of rabbits with phenobarbital. We have now shown that this is also the case for form 5.

Phenobarbital has no effect on the concentrations of form 2 or form 5 in the lung. In the liver, the induction of form 5 by phenobarbital has been shown by single radial immunodiffusion, SDS-polyacrylamide gel electrophoresis, antibody inhibition of enzyme activity and increased metabolism of aromatic amines to mutagenic products.

2) The pathways of activation of 2-acetylaminofluorene differ in rabbit liver and lung -- Acetylaminofluorene (2-AAF) is generally thought to be metabolized to mutagenic products by first being N-hydroxylated to N-OH-AAF and then deacetylated to N-OH-AF. Rabbit pulmonary microsomal fractions are more efficient at catalyzing the activation of 2-AAF to mutagenic product(s) than are similar fractions from liver. This difference is due to the higher concentration of cytochrome P-450, form 5, in the lung. The profile of metabolites -- determined by HPLC -- formed from 2-AAF in pulmonary incubations does not include N-OH-AAF. The steps in the pathway for metabolism of 2-AAF in the rabbit lung has been determined to be: 1) deacetylation of 2-AAF to 2-AF; 2) N-hydroxylation of 2-AF to N-OH-AFF. In the liver N-OH-AAF is formed but the major metabolite is 2-AF. The activation of 2-AAF via N-OH-AAF appears to be a minor route of metabolism in liver and does not occur in lung. The activation of aromatic amines in the liver is greatly increased following treatment of rabbits with phenobarbital. This increase is due to the induction of cytochrome P-450, form 5.

3) The concentrations of the pulmonary isozymes of cytochrome P-450 (forms 2, 5 and 6) can be modulated by treatment of rabbits with various chemicals -- Induction of the synthesis of various isozymes of cytochrome P-450 in liver can be brought about by a number of chemicals. The effects of these chemicals on the pulmonary cytochrome P-450 system are not the same. For example, PCB's increase the cytochrome P-450 concentration in liver but decrease it in lung. The effect in the lung appears to be due to the repression of the synthesis of form 2, which makes up 40% of the pulmonary P-450. In the liver, the same effect occurs but involves only about 5% of the total P-450. The primary effect in the liver is the induction of form 4 which does not take place in the lung. Form 4 is also induced in the liver by TCDD as is form 6. In the lung only form 6 is induced by TCDD. Phenobarbital, a potent inducer of forms 2 and 5 in the liver, does not induce in the lung. However, treatment of rabbits with phenobarbital is followed by a depression of the level of form 6 in the lung. In addition, the induction of form 6 in the lung by the co-planar isomers of PCB's can be blocked by phenobarbital.

The control of the synthesis of the several isozymes of cytochrome P-450 in rabbit lung will be investigated. Particular attention will be paid to the interactions of various classes of inducers. The role of each isozyme in the metabolism of exogenous chemicals will be investigated. New techniques of molecular biology and protein chemistry will be utilized for these studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Through a detailed and complete characterization of the rabbit pulmonary cytochrome P-450-dependent monooxygenase system, major differences between the abilities of the liver and lung to metabolize and, in some cases, activate exogenous chemicals, are being elucidated. For example, it is now clear that the Clara cells of the lung contain a higher concentration of the enzymes required for the activation of 4-ipomeanol than do hepatocytes. This finding may explain the pulmonary specificity of this toxin. In addition, this research has provided both information (for example, substrate specificities of different isozymes) and preparations (antibody preparations and purified enzymes) that have been very useful to furthering the research efforts of other investigators at NIEHS.

PUBLICATIONS

Robertson, I. G. C., Philpot, R. M., Zeiger, E., and Wolf, C. R.: Specificity of rabbit pulmonary cytochrome P-450 isozymes in the metabolism of several aromatic amines and aflatoxin B_1 to mutagenic products. Molec. Pharmacol. 20: 662-668, 1981.

Devereux, T. R., Serabjit-Singh, C. J., Slaughter, S. R., Wolf, C. R., Philpot, R. M., and Fouts, J. R.: Identification of pulmonary cytochrome P-450 isozymes in nonciliated bronchiolar epithelial (Clara) and alveolar type II cells isolated from rabbit lung. Exp. Lung. Res. 2: 221-230, 1981.

Philpot, R. M., Wolf, C. R., Slaughter, S. R., Bend, J. R., Robertson, I. G. C., Zeiger, E., Statham, C. N., and Boyd, M. R.: The role of the cytochrome P-450-dependent monooxygenase system in pulmonary specific toxic effects of xenobiotics. Fifth International Symposium on Microsomes and Drug Oxidations, Tokyo, Japan. In press.

Philpot, R. M., and Wolf, C. R.: The properties and distribution of the enzymes of pulmonary cytochrome P-450-dependent monooxygenase systems. In Hodgson, E., Bend, J. R., and Philpot, R. M. (Eds.): Reviews in Biochemical Toxicology 3. New York, Elsevier/North Holland, 1981, pp. 51-76.

Wolf, C. R., Statham, C. N., McMenamin, M. G., Bend, J. R., Boyd, M. R., and Philpot, R. M.: The relationship between the catalytic activities of rabbit pulmonary cytochrome P-450 isozymes and the lung specific toxicity of the furan derivative, 4-ipomeanol. Molec. Pharmacol. In press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80002-12 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodora R. Devereux	Research Biologist	LP	NIEHS
	James R. Fouts	Research Pharmacologist	LP	NIEHS
Other:	Richard M. Philpot	Research Chemist	LP	NIEHS
	Cosette Serabjit-Singh	Chemist	LP	NIEHS
	Kenneth Jones	Visiting Fellow	LP	NIEHS
	John R. Bend	Chief	LP	NIEHS
	Tore Aune	Postdoctoral Research Fellow	LP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION
NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	1.5	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range purpose of this project to study how various chemicals and physiological changes affect xenobiotic metabolism by the body. This laboratory has concentrated its effort on the lung as a target organ for exposure to environmental stresses. Present studies include isolation of rabbit lung cell types for the purpose of studying localization of xenobiotic metabolism within the lung and toxication-detoxication mechanisms in individual cell populations. The following model enzyme systems are being used for study of individual xenobiotic metabolic pathways in lung cell populations: coumarin hydroxylase, 7-ethoxycoumarin deethylase, benzo(a)pyrene hydroxylase, epoxide hydrolase, and glutathione transferase. Different lung cell fractions (mixed cell populations) appear to have different metabolic profiles indicating possible differences in cytochrome content in the cell types. Mixed-function oxidase activity is now being studied and compared in cell fractions containing either 80% alveolar type II cells or 70% nonciliated bronchiolar epithelial cells (Clara cells).

PROJECT DESCRIPTION

METHODS EMPLOYED: Protease type I (Sigma) instilled into the trachea is used for dispersal of rabbit lung cells. Populations of cells are separated from the cell digest according to their rates of sedimentation by the technique of centrifugal elutriation. Other methods employed for cell separation include density gradients, phase separation, differential attachment to tissue culture plates, and affinity chromatography. Cells are studied using light microscopic (including fluorescence microscopy) and electron microscopic techniques. Spectrophotometric, fluorometric, and radiometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques have been developed to disperse and separate the many lung cell types in order to localize and study drug metabolism in individual cell populations. Past research was directed strongly toward obtaining relatively pure populations of alveolar type II cells (80-90% purity) and Clara cells (60-70% purity) since these cell types contain a majority of the pulmonary endoplasmic reticulum (where mixed-function oxidase activity seems to occur). The alveolar type II cell fraction contains 7-ethoxycoumarin (7-RC) deethylase, benzo(a)pyrene hydroxylase, epoxide hydrolase and glutathione transferase activities, although little coumarin hydroxylase activity has been observed. All these activities are greatly enriched in the Clara cell fraction (60-70% purity) and are being compared to what was found in the type II cells as well as in whole lung homogenate. Immunological techniques with cell fractions and antibodies to the purified cytochromes have been used to localize the rabbit pulmonary cytochromes $P\text{-}450_2$ and $P\text{-}450_5$ (formerly called $P\text{-}450_I$ and $P\text{-}450_{II}$, respectively) in the separate lung cell populations. With immunohistochemical methods and SDS-polyacrylamide gel electrophoresis, we have demonstrated that both cytochromes $P\text{-}450_2$ and $P\text{-}450_5$ are present in the isolated type II and Clara cells. Metabolism and covalent binding of 4-ipomeanol, a pulmonary toxin with specific in vivo Clara cell toxicity, has also been studied in the isolated cells. Metabolic activation and covalent binding were observed in both the isolated Clara and type II cells, although to a much greater extent in the Clara cells. Techniques to prevent loss of monooxygenase activity during cell isolation and culture have also been investigated. One mM nicotinamide and 10 mM fructose used in the protease and cell isolation buffers increased 7-EC deethylase activities significantly in the type II cells and variably in the Clara cells. Electron flow in metabolism of 7-EC and p-nitroanisole (p-NA) in Clara and type II cells has been examined with some differences noted between the two cell types. There was more NADH metabolism of 7-EC and p-NA in Clara than in type II cells. Also, the antibody of cytochrome $P\text{-}450_2$ inhibited only 60-70% of 7-EC and p-NA dealkylation in Clara cell microsomes, but it inhibited 90% of the metabolism in the type II cell microsomes indicating the possibility of another unidentified cytochrome P-450 isozyme in the Clara cells. The identification of this cytochrome P-450 isozyme is being investigated. The metabolism of acetylaminofluorene (AAF) in type II and Clara cell microsomes is being studied in collaboration with Drs. T. Aune and J. Bend. Future research will also focus on the metabolism by cytochrome P-450 of endogenous substrates such as arachidonic acid and testosterone.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Use of lung cells to study drug metabolism may lead to a better understanding of lung damage and repair mechanisms. Comparisons will be made of cellular versus microsomal, purified, and isolated perfused lung xenobiotic metabolism systems to see where differences exist and what this may contribute to understanding toxication and detoxication mechanisms in the body. Studies of xenobiotic metabolism in lung cell populations may give us a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals and physiological stresses can alter these systems and this balance.

PUBLICATIONS

Devereux, T. R. and Fouts, J. R.: Xenobiotic metabolism by alveolar type II cells isolated from rabbit lung. Biochem. Pharmacol. 30: 1231-1237, 1981.

Devereux, T. R. and Fouts, J. R.: Isolation of different pulmonary cells and subsequent study of their xenobiotic/drug-metabolizing enzyme systems. In Jakoby, W. (Ed.): Methods in Enzymology. New York, Academic Press, 1981, Vol. 77, pp. 147-154.

Devereux, T. R., Serabjit-Singh, C. J., Slaughter, S. R., Wolf, C. R., Philpot, R. M. and Fouts, J. R.: Identification of cytochrome P-450 isozymes in nonciliated bronchiolar epithelial (Clara) and alveolar type II cells isolated from rabbit lung. Exp. Lung Res. 2: 221-230, 1981.

Devereux, T. R. Jones, K. G., Bend, J. R., Fouts, J. R., Statham, C. N. and Boyd, M. R.: In vitro metabolic activation of the pulmonary toxin, 4-ipomeanol, in nonciliated bronchiolar epithelial (Clara) and alveolar type II cells isolated from rabbit lung. J. Pharmacol. Exp. Ther. 220: 223-227, 1982.

Devereux, T. R.: An integrated approach to studying xenobiotic metabolism in lung: Importance of investigation in Clara and alveolar type II cells. Federation Proceedings, In Press.

Bend, J. R., Smith, B. R., Ball, L. M., Plummer, J. L., Wolf, C. R., Philpot, R. M., Devereux, T. R. and Fouts, J. R.: Metabolism of benzo(a)pyrene and benzo(a)pyrene 4,5-oxide in rabbit lung. In Snyder, et al. (Ed.): Biological Reactive Intermediates. Plenum Publishing Corporation, 1982, Vol. II, Part A, pp 541-554.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-09 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Xenobiotic-Metabolizing Enzyme Activity in Skin and It's Response to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Roberta J. Pohl	Research Biologist	LP	NIEHS
	James R. Fouts	Research Pharmacologist	LP	NIEHS
	Marguerite Coomes	Staff Fellow	LP	NIEHS
Other:	Rebecca Sparks	Biological Technician	LP	NIEHS
	Fred Talley	Head, Histology	TRTP	NIEHS
	Michael Boyd	Head, Molecular Pharmacology	LCPB	NCI
	Charles N. Statham	Cancer Expert	LCPB	NCI

COOPERATING UNITS (if any)
Biometry Branch, Histology

LAB/BRANCH
Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION
NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.2	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to elucidate the role of xenobiotic-metabolizing enzymes in skin as mediators of the toxicity of environmental agents. Mixed-function oxidases, (including aryl hydrocarbon hydroxylase), glutathione S-transferase, UDP-glucuronosyltransferase and sulfotransferase activities are measured in whole skin, epidermal cells or subcellular fractions of epidermal cells from hairless mice (Hrs/J). Mixed-function oxidase activities in Zymbal's glands from mice and rats were compared with those in skin sebaceous cells. Epidermal cell types high in xenobiotic metabolizing activity and/or cytochrome P-450 content are being identified. Changes in xenobiotic metabolism and/or in the content of mixed-function oxidase components after exposure (topical or systemic) of mice to various effectors such as ultraviolet radiation, polycyclic hydrocarbons, and steroids are being investigated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Epidermal cells were isolated by digestion with pronase and then separated into different populations by density gradient centrifugation and elutriation. Cells were homogenized in a French Press at 10,000 psi for some assays. Sebaceous cells were identified histologically by fluorescence of Nile blue sulfate-stained neutral lipids.

MAJOR FINDINGS AND PROPOSED COURSE: Epidermal and sebaceous cells (> 75% viable), freed from the skin of hairless mice by digestion (at 37°C) with pronase, were separated into different cell fractions by metrizamide gradient centrifugation. The fraction containing about 60% of the cells (a population containing viable cells in all stages of differentiation) was separated into eight fractions by elutriation. Some fractions which were recovered from the elutriator were enriched (up to 70%) in basal cells. 7-Ethoxycoumarin O-deethylation, the average cell volume, and the protein to DNA ratio all increase in later fractions. Papanicolaou-stained slides of the fractions were examined. The earlier fractions contain mostly basal cells and cells in early stages of differentiation. The later fractions are rich in more fully differentiated keratinocytes. These findings confirm our interpretation of earlier results with less pure fractions that basal cells are low in 7-ethoxycoumarin O-deethylation activity relative to the total cell population or the differentiated keratinocyte population.

The total population of epidermal and sebaceous cells isolated was used to study the interaction of oxidative (cytochrome P-450-dependent) and conjugating (glucoronidation and sulfation) xenobiotic metabolizing activities. Kinetic models were derived to show the interrelationship of the two pathways, and the relative contribution of free, glucuronidated and sulfated metabolites to the total metabolic product evaluated. Further studies are planned with the kinetic model and to investigate the role of cofactor availability in modulation of xenobiotic metabolism in the epidermis.

In earlier work, the sebaceous cell population had high 7-ethoxycoumarin O-deethylation, benzo(a)pyrene hydroxylation and conjugation activities. We examined sebaceous tissue from mouse skin (two fractions, one containing a greater proportion of terminally differentiated cells), rat skin, and rat and mouse Zymbal's gland. The monooxygenase activity of this tissue also appears to be related to the relative stage of differentiation, terminally differentiated tissue having low activity relative to that of tissue in early stages of differentiation.

In the future we will characterize the epidermal cell population isolated by pronase for its metabolic status and the stability of this status with time in the first few hours after isolation. In particular, we will determine the stability of the cytochrome P-450-dependent monooxygenase system. We will then study the substrate specificity of the monooxygenase and conjugation systems of basal-, sebaceous- and keratinocyte-enriched fractions and this may indicate enzyme isozyme distribution in the cell types. We will investigate the metabolism of selected steroids by isolated cells since skin function changes in response to changes in systemic hormone levels. In addition, we will examine the effects of various inhibitors of cytochrome P-450 isozymes and other enzymes which might metabolize 4-ipomeanol on the autoradiographic demonstration of 4-ipomeanol metabolites in skin slices. In this way we may

determine the cause for our observation that piperonyl butoxide does not prevent such binding.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Persistent environmental pollutants, specifically chlorinated organic compounds, are often accumulated in skin. Many other biologically active chemicals are applied to the skin in medicaments, cosmetics, cleaning, compounds, etc. Xenobiotic-metabolizing enzymes in the skin may have a role in the locally or systemically expressed toxicity of these compounds. Increased understanding of that role may lead to development of better systems to assess toxicity of chemicals. Manipulation of xenobiotic metabolism may be used to maximize the beneficial effects of chemicals applied to the skin while minimizing toxic reactions.

PUBLICATIONS

Coomes, M. W., Norling, A. H., Pohl, R. J., Müller, D. and Fouts, J. R.: Foreign compound metabolism by isolated skin cells. Cancer Res., in press.

Pohl, R. J., Serabjit-Singh, C. J., Slaughter, S. R., Albro, P. W., Fouts, J. R. and Philpot, R. M.: Hepatic microsomal NADPH-cytochrome P-450 reductase from little skate, Raja erinacea. Comparison of thermolability and other molecular properties with mammalian enzyme. Arch. Biochem. Biophys.. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80005-09 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

In Vitro Metabolism of Xenobiotics by Selected Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Bend	Chief, LP	LP	NIEHS
Other:	Gary L. Foureman	Biologist	LP	NIEHS
	Richard M. Philpot	Research Chemist	LP	NIEHS
	Cosette Serabjit-Singh	Research Chemist	LP	NIEHS
	Oscar Hernandez	Senior Staff Fellow	LP	NIEHS
	Shelley Slaughter	Senior Staff Fellow	LP	NIEHS

COOPERATING UNITS (if any)

Biometry Branch; Laboratory of Environmental Chemistry; C. B. Whitney Marine Laboratory, University of Florida

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.5	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the biotransformation of foreign organic compounds in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Both cytochrome P-450-dependent monooxygenase activities and alkene and arene oxide-metabolizing enzymes (epoxide hydrolase and glutathione transferases) are being characterized in control fish and in fish pre-exposed to polycyclic aromatic hydrocarbons. Some studies are being undertaken with purified cytochrome P-450 and with purified glutathione transferases from aquatic species. Emphasis is given to projects that will enhance our understanding of toxication-detoxication mechanisms.

PROJECT DESCRIPTION

OBJECTIVES: 1. To characterize the hepatic monooxygenase (MO) system and the glutathione transferases of representative species from Maine and Florida, before and after pretreatment with polycyclic hydrocarbon-type inducing agents.

2. To examine the MO components and membrane dynamics of little skate (Raja erinacea) hepatic microsomes in order to explain the greater thermolability, during in vitro assay, of hepatic MO from skate when compared to that from rabbit.

METHODS EMPLOYED: Differential centrifugation; enzyme purification; spectroscopic, fluorometric, and radiochemical assays; organ perfusion procedures; ion exchange; thin-layer, paper, high-pressure liquid chromatography; and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Four distinct glutathione transferases were isolated from the liver of the little skate, Raja erinacea, that efficiently catalyze the reaction of benzo(a)pyrene 4,5-oxide with glutathione. Three other K-region polycyclic arene oxides (pyrene 4,5-oxide, benz(a)anthracene 5,6-oxide and phenanthrene 9,10-oxide) were also shown to be very good substrates for the major transferase, E-4. This enzyme was shown to be stereospecific with each of the polycyclic arene oxide substrates; that is, only one of each possible pair of diastereomeric glutathione adducts was formed in each case. With benzo(a)pyrene 4,5-oxide, enzymatic attack of the sulfur atom of glutathione occurs only at the carbon atom of the arene oxide having R configuration. Based on similarities in HPLC elution characteristics this is presumed, but not proven, to be true also with pyrene 4,5-, phenanthrene 9,10- and benzo(a)anthracene 5,6-oxide. The four transferases of little skate (E-2 through E-4) show a strong preference for the (-)-4R,5S-enantiomer of benzo(a)pyrene 4,5-oxide relative to the (+)-5S,4R-enantiomer. The ultimate carcinogen trans-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene was also a substrate for E-4 although the turnover of this compound was approximately two orders of magnitude less than with benzo(a)pyrene 4,5-oxide. Future work will kinetically characterize the transferase E-4 catalyzed reaction between glutathione and the four polycyclic K-region oxides described here; this work will include product inhibition studies. In addition, the enzymatic reaction of benzo(a)pyrene 7,8-oxide, benzo(a)pyrene 9,10-oxide (non-K-region polycyclic arene oxides) and the 7,8-dihydrodiol-9,10-epoxides of benzo(a)pyrene with glutathione will also be characterized using skate transferase E-4.

2. The monomeric molecular weights, obtained by SDS gel electrophoresis of purified skate and rabbit NADPH-cytochrome P-450 reductase were 74,000 and 72,000 daltons, respectively. Further partial proteolysis of the reductases yielded peptides of dissimilar monomeric molecular weights, suggesting significant structrual differences. In spite of these differences, the antibody to the rabbit enzyme partially inhibited the reduction of cytochrome c by the skate reductase, but no cross-reactivity upon Ouchterlony double immunodiffusion was observed. The skate reductase supported the rabbit cytochrome P-450 form 2-mediated N-demethylation of benzphetamine, but not to the same extent as did the rabbit reductase.

The unusual redox behavior of the flavins of the skate reductase, different from that reported for mammalian reductases, is to be further investigated to determine

to what extent, if any, it affects the support of cytochrome P-450-mediated reactions or reflects the thermolabile nature of the protein. In this regard, a comparison of the skate reductase with that of Atlantic stingray NADPH-cytochrome P-450 reductase, the heat stability of which is very similar to that of the rabbit, is underway.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products, and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food web, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and hazard as direct and indirect foodstuffs for man. Comparison of the enzymes involved in metabolizing xenobiotics in different species should lead to a better mechanistic understanding of the overall effects of xenobiotics on animals, and of animals on xenobiotics.

Moreover, a detailed understanding of metabolic activation and deactivation pathways in marine animals may allow us to predict those species most likely to be affected by carcinogens, mutagens, teratogens, or cytotoxins in the environment and allow us to use them as sentinel or early warning indicators of toxic environmental contaminants.

PUBLICATIONS

Foureman, G. L., D'Amico, C., Vom Scheidt, A. T., Fouts, J. R., and Bend, J. R.: Changes in the hepatic cytochrome P-450-dependent monooxygenase system of winter flounder following treatment with polycyclic aromatic hydrocarbons. Bull. Mt. Desert Island Biol. Lab. 20: 132-136, 1980.

Serabjit-Singh, C. J., Bend, J. R., and Philpot, R. M.: Purification of hepatic microsomal NADPH-cytochrome P-450 reductase of little skate, Raja erinacea. Bull. Mt. Desert Island Biol. Lab. 21: 57-59, 1981.

Little, P. J., James, M. O., Bend, J. R., and Ryan, A. J.: Imidazole derivatives as inhibitors of cytochrome P-450-dependent oxidation and activation of epoxide hydrolase in hepatic microsomes from a marine fish. Biochem. Pharmacol. 30: 2876-2880, 1981.

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(±)-benzo[a]pyrene 4,5-oxide. In Polynuclear Aromatic Hydrocarbons: Fifth International Symposium on Chemical Analysis and Biological Fate, Columbus, Battelle Press, 1981, pp. 667-674.

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Bend, J. R., James, M. O., Little, P. J., and Foureman, G. L.: In vitro and in vivo metabolism of benzo[a]pyrene by selected marine crustacean species. In Dawe, C. J. et al. (Eds.): Phyletic Approaches to Cancer, Tokyo, Japan, Sci. Soc. Press, 1981, pp. 179-194.

Foureman, G. L., and Bend, J. R.: The capabilities of fish and other aquatic organisms for xenobiotic metabolism. In Proceedings, Fifth International Congress of Pesticide Chemistry, Kyoto, Japan, August 29-September 4, 1982.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80007-11 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Bend	Chief, LP	LP	NIEHS
Other:	Craig Harris	"P" Appointment	LP	NIEHS
	Gary L. Foureman	Biologist	LP	NIEHS
	Richard M. Philpot	Research Chemist	LP	NIEHS
	Oscar Hernandez	Visiting Associate	LEC	NIEHS
	Tore Aune	Research Fellow	LP	NIEHS

COOPERATING UNITS (if any)
Biometry Branch; Laboratory of Environemental Chemistry

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION
NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.4	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several arene and alkene oxides are known to react covalently with macromolecules, including nucleic acids, and to transform cells in vitro, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the cytochrome P-450-dependent monooxygenases, which convert unsaturated hydrocarbons to epoxides, and the further metabolism of arene and alkene oxides by soluble fraction glutathione transferases and microsomal epoxide hydrolase in hepatic and extrahepatic tissues. The relative quantitative importance of these metabolic pathways is being studied at various levels of cellular organization (isolated cells, perfused organs, purified enzymes) in an attempt to understand the mechanisms of organ-specific and cell-specific toxicity mediated by compounds metabolized to epoxides.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1) To characterize in intact tissue the enzymatic pathways responsible for the metabolic conversion of polynuclear aromatic hydrocarbons to epoxides and for the decomposition of these reactive intermediates; 2) to assess the importance of these enzyme systems in protecting tissues from reactive intermediates; 3) to evaluate the influence of cellular integrity and intracellular location of enzymes on the metabolism of reactive intermediates; 4) to correlate dosage levels with activity of epoxide-metabolizing pathways and toxicity (including histopathology and covalent binding of radioactivity to macromolecules) and rates of excretion in isolated perfused organs; and 5) to study the regioselectivity and stereoselectivity of various glutathione transferases with selected alkene and arene oxide substrates.

METHODS EMPLOYED: Purified enzymes, subcellular fractions of homogenates from various tissues, isolated perfused livers, kidneys, and lungs, isolated cells and intact animals are used to study alkene and arene oxide formation, biotransformation and toxicity. Highly purified radiolabeled substrates are used in most experiments. Metabolite isolation, identification and quantitation are most frequently accomplished using high pressure liquid chromatography (HPLC) and scintillation counting. Detailed characterization (^{13}C-NMR, NMR, mass spectroscopy, etc.) and synthesis of metabolites, when required, are routinely accomplished in collaboration with the Laboratory of Environmental Chemistry (LEC). In studies investigating the relationships between biotransformation and toxicity total metabolic profiles are determined and covalent binding to RNA, DNA and protein is measured, but only as a crude index of the amount of reactive metabolite present/formed.

MAJOR FINDINGS AND PROPOSED COURSE: 1. The stereo- and regioselectivity of purified rat liver glutathione transferases were studied using ^{3}H-R- and ^{3}H-S-styrene 7,8-oxide. Specific activities and kinetic constants suggest that stereoselectivity is a function of the individual glutathione transferase isozyme. Regioselectivity studies with racemic substrate have shown that the preferential formation of one of the two possible diastereomeric products (for each enantiomer) is a function of the styrene oxide enantiomer used as substrate and its concentration. Product inhibition studies are in progress to help elucidate the mechanism of enzymatic reaction between glutathione and the two sytrene 7,8-enantiomers in the presence of various rat liver transferases. The work will be extended to include various polycyclic K-region oxides, including benzo(a)pyrene 4,5-oxide, benz(a)anthracene 5,6-oxide, pyrene 4,5-oxide and phenanthrene 9,10-oxide, and the pulmonary and hepatic glutathione transferases purified from rabbit.

2. Using an HPLC system that resolves the four possible diastereomeric glutathione conjugates of styrene 7,8-oxide, the stereochemistry of the cytochrome P-450-dependent oxidation of styrene by hepatic microsomes from untreated rats was studied. The styrene 7,8-oxide formed during the incubation period is almost totally (more than 95%) converted to the corresponding glutathione adducts by the inclusion of glutathione transferases, glutathione and cyclohexene oxide, an epoxide hydrolase inhibitor, in the incubation mixture. Results to date demonstrate a preference (1.2- to 1.5-fold) for the formation of S-styrene 7,8-oxide relative to R-styrene 7,8-oxide. Microsomal preparations from rats induced by the administration of phenobarbital or 3-methylcholanthrene are currently being assessed for stereoselectivity in the P-450-dependent oxidation of styrene. These experiments will be

extended to reconstituted monooxygenase systems containing purified pulmonary and hepatic cytochrome P-450 from the rabbit and to purified rat hepatic cytochromes P-450 (the rat studies will be done in collaboration with Dr. Fred Guengerich, Vanderbilt University).

3. Earlier studies in the Laboratory of Pharmacology demonstrated that 2-aminofluorene was converted to mutagenic metabolites by rabbit pulmonary microsomes much more efficiently than by rabbit hepatic microsomes. As this was not true for 2-acetylaminofluorene (2-AAF), the metabolism of 2-AAF was studied in rabbit pulmonary and hepatic microsomal preparations. 2-Aminofluorene (AF) was the major 2-AAF metabolite in microsomes from both tissues. Further oxidative metabolism of 2-AF was one order of magnitude more rapid in pulmonary than in hepatic microsomes on a cytochrome P-450 basis. Microsomes prepared from isolated Clara and alveolar type II cells of rabbit lung also metabolized 2-AAF to the same oxidative products formed with pulmonary microsomes. Clara cells were more active at catalyzing this reaction, consistent with their higher cytochrome P-450 content. The major carbon oxidation product formed from 2-AAF by both hepatic and pulmonary microsomes was 7-OH-2-AAF. Antibodies to rabbit cytochrome P-450 form 5 inhibited the 7-hydroxylation of 2-AAF only about 40% in rabbit lung microsomes. As cytochrome P-450 form 2, the other major form of cytochrome P-450 in rabbit lung, is reported to be inactive in the metabolism of 2-AAF, these data suggest the presence of additional cytochrome(s) in lung microsomes that are active in the C-oxidation of 2-AAF.

Further studies characterizing the metabolism of 2-AAF in reconstituted monooxygenase systems containing rabbit pulmonary cytochrome P-450 form 2 and form 5 are currently underway. Studies with inhibiting antibodies to these two forms of cytochrome P-450 will also be conducted on 2-AAF metabolism in microsomes prepared from rabbit pulmonary Clara cells and alveolar type II cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Xenobiotics or their chemically reactive metabolic intermediates (e.g., alkene or arene oxides) are detoxified by several pathways, principally involving conjugation in liver, lung, gut, and other extrahepatic tissues, and excreted from the animal. The activities of these conjugation and excretory mechanisms may be important factors in determining the susceptibility of organs, or individuals, to environmental agents. A detailed understanding of the pharmacological, biochemical and chemical aspects of these toxication and detoxication processes should result in our ability to protect certain individuals from chemical insult.

In the real-life situation one is very rarely exposed to a single xenobiotic; rather, one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, hydrocarbons, etc. For this reason investigations concerning interactions between more than one foreign compound that share a common metabolic pathway are of relevance to environmental health.

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Steele, J. W., Yagen, B., Hernandez, O., Cox, R. H., Smith, B. R., and Bend, J. R.: The metabolism and excretion of styrene oxide-glutathione conjugates in the rat by isolated perfused liver, lung and kidney preparations. J. Pharmacol. Exptl. Therap. 219: 35-41, 1981.

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Leakey, J. E. A., Mukhtar, H., Fouts, J. R., and Bend, J. R.: Thyroid hormone-induced changes in the hepatic monooxygenase system, heme oxygenase activity and epoxide hydrolase activity in adult male, female and immature rats. Chem.-Biol. Interactions. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80031-06 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Role of Altered Membrane Function in Xenobiotic Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Pritchard	Research Physiologist	LP	NIEHS
Other:	Soon-Ho Lee	Expert Research Biochemist	LP	NIEHS
	J. Larry Renfro	IPA Research Physiologist	LP	NIEHS

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Pharmacology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.4	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many aquatic animals are highly sensitive to specific xenobiotics. Such organisms are used as models to identify those physiological processes most sensitive to environmental pollutants. Since the exposed location and functional importance of cell membranes make them particularly susceptible to the toxic effects of foreign chemicals, we have focused primarily on the interactions of xenobiotics with membrane transport systems particularly transport in epithelia where the coupled function of apical and basolateral membranes provides the basis of physiological control mechanisms. We have used kidney and gill since (1) function of these organs depends in large part on membrane transport, (2) they both play important roles in determining the rate at which many foreign compounds are excreted from the body, and (3) each is vital to the overall homeostasis of the organism.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1. To characterize membrane permeability and transport in organs (e.g. kidney, intestine, gill) where these factors play a critical role in organ function.

2. To evaluate the hypothesis that alteration of membrane function by foreign chemicals may lead to disruption of physiological systems dependent upon such function.

3. To determine if such disruption plays a significant role in the toxicity of a given pollutant.

METHODS EMPLOYED: Kidney: Vesicles are prepared from flounder kidney lumenal membranes (BBM) by Ca^{++} precipitation and differential centrifugation. Basolateral membrane (BLM) vesicles are prepared by differential centrifugation followed by density gradient centrifugation. Transport into these vesicles is assessed using millipore filtration techniques. In both cases, we take advantage of two important factors by using the flounder. First, flounder kidney consists almost exclusively of proximal tubules; thus, we have a more homogenous population of nephrons from which to prepare membrane vesicles. Second, the flounder tubule may be readily studied in vitro (isolated tubules) and in vivo (clearance techniques); thus, results obtained from isolated membranes may be compared directly with intact cell and tubule function.

Gill: Upon exposure of blue crabs to low salinity, ion transport and gill ATPase increase markedly. This provides a system in which animals are already stressed and may be particularly sensitive to impairment of the membrane events required for ionic and osmotic regulation. Both Na^+, K^+-ATPase and HCO_3^--ATPase are assessed in microsomes prepared by polytron disruption and differential centrifugation of gill tissue. These enzymes have now been solubilized in Triton X-100 and will be reconstituted in proteoliposomes to examine both the basic mechanisms of transport and the interactions of the ATPase-associated ion fluxes with xenobiotics.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Renal transport-- We have focused on two reabsorptive transport systems localized on the lumenal membrane (BBM) of the renal tubule, glucose and basic amino acid. While these systems have analogous roles, conservation of filtered substrate, their mechanisms are quite different. Kinetic studies in BBM vesicles using equilibrium techniques have shown that 1) glucose reabsorption is driven by the electrochemical gradient for sodium, 2) it is electrogenic, and 3) it is highly sensitive to inhibitors, such as mercury, which bind to the carrier or ionophores which dissipate the sodium gradient. On the other hand, the basic amino acid, L-lysine, is reabsorbed in the teleost kidney via a proton gradient driven system. It is not sodium dependent. It is therefore sensitive to agents like dinitrophenol, FCCP, or pentachlorophenol which act as proton ionophores to dissipate the proton gradient. This system (basic amino acid) is unique. Other amino acids are reabsorbed via Na-dependent systems analogous to the glucose system.

The second major system studied was sulfate transport. Sulfate, a divalent anion, is secreted by the teleost. We have shown that secretion is mediated by a pH

gradient driven, electroneutral uptake at the BLM and inhibited by mercury and protonophores. Because the electrochemical gradient at that pole of the tubular cell is steep, this mechanism effectively drives sulfate into the cell. Exit at the apical pole of the cell (BBM) is mediated by an anion exchanger. Bicarbonate is the most effective counter ion, but chloride and thiocyanate may substitute. The kinetics of the exchanger appear to fit a ping-pong mechanism in which the carrier is first loaded at one face of the membrane, is translocated to the other face, the first anion dissociates and is replaced by a second anion which is moved in the opposite direction. Many of the properties of the exchanger are similar to the anion exchange system of red cells. However, the renal system is distinguished by its relatively poor affinity for chloride and its response to several inhibitors. Based on these results we hypothesized that in mammals, where sulfate is reabsorbed rather than secreted, there might be such an exchanger on the BLM. This proved to be the case, we are currently characterizing this system. Already it is clear that, like the teleost exchanger, the mammalian carrier functions most effectively with bicarbonate as the counter ion.

Major future emphasis will be on the functional roles of this anion exchanger which appears to be widely distributed and which may play important roles in volume and pH regulation as well as ion transport in these tissues. In addition, preliminary evidence indicates that at least one step in the organic anion transport system (the major system mediating excretion of xenobiotics and their metabolites) is mediated by such an exchange process.

2. Gill transport -- While major emphasis this year has been on renal function, we have also continued our work on ion transport in crustacean gill. We have used differential and gradient centrifugation to examine the localization of the HCO_3^--ATPase in the blue crab gill. Some workers have suggested that this enzyme is exclusively mitochondrial in origin; thus, that it does not directly mediate HCO_3^- or Cl^- transport. Our results demonstrated quite clearly that a significant fraction of HCO_3^--ATPase activity is associated with the plasma membrane in preparations essentially free of mitochondrial contamination. This result, in conjunction with the earlier demonstration of close correlation of HCO_3^--ATPase activity with ion transport during dilute seawater adaptation, is certainly consistent with a role for this enzyme in anion transport. Major future emphasis will be on reconstitution of the HCO_3^--ATPase system in liposomes where its transport role may be more readily evaluated. This system should also provide an optimal setting in which to evaluate effects of xenobiotics on ion transport and osmoregulation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of their functional importance and their exposure to the full extracellular concentration of potentially toxic chemicals, cell membranes are particularly susceptible to the effects of xenobiotics. Preparations such as the isolated flounder tubule and the BBM and BLM vesicles derived from those tubules offer unique opportunities to examine the development and mechanisms of such membrane toxicity. Furthermore, since many marine organisms are particularly sensitive to certain agents, e.g., the blue crab to organochlorines, these studies may also permit identification of systems particularly prone to disruption by environmental contaminants. Such studies may then (a) point to sites which might also be prone to damage in man and (b) serve as indicators or warning systems for the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants.

PUBLICATIONS

Pritchard, J.B., Booz, G. and Kleinzeller, A.: Renal sugar transport in the winter flounder VI Reabsorption of D-mannose. Am. J. Physiol. 242: F415-F422, 1982.

Squibb, K.S., Pritchard, J.B. and Fowler, B.A.: Renal metabolism and toxicity of metallothionein. Proceedings on the Biological Function of Metallothionein. Elsevier, New York, pp. 181-192, 1982.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80032-06 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Excretion and Toxicity of Xenobiotics to Marine and Terrestrial Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John B. Pritchard Research Physiologist LP NIEHS

COOPERATING UNITS (if any)

Dr. L. O'Tuama, Dept. of Neurology, Univ. North Carolina, School of Medicine, and Dr. A. L. Krall, Dept. of Biochemistry, Medical University of South Carolina.

LAB/BRANCH
Laboratory of Pharmacology

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Marine and terrestrial vertebrates are used to examine the role of organic anion transport in the renal and hepatic excretion of environmental contaminants such as DDT, 2,4-dichlorophenoxyacetic acid (2,4-D), and the polycyclic aromatic hydrocarbon, benzo(a)pyrene (BP). Topics under investigation include examination of 1) the importance of renal and hepatic organic anion transport in the rate of elimination of xenobiotics or their metabolites; 2) the interference of foreign compounds with elimination of endogenous wastes or toxins; 3) the role of intracellular binding proteins, such as glutathione S-transferases, in transport and toxicity of organic ions; 4) the influence of metabolism on the route and rate of xenobiotic excretion; and 5) the impact of membrane transport-related cellular accumulation in the development of the xenobiotic toxicity in target organs. The role of transport in the elimination of xenobiotics from specific organs, e.g., brain, as well as from the whole organism, is also characterized.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1. To evaluate the factors which determine the rate of xenobiotic excretion. These include active transport, metabolism, plasma binding, and intracellular binding.

2. To assess the consequences of the extensive accumulation of xenobiotics resulting from organic anion transport in the kidney and other organs possessing this transport system.

3. To determine the mechanisms leading to the toxicity of foreign compounds, with particular emphasis on the interactions of such compounds with membrane function.

METHODS EMPLOYED: Our primary approach is comparative. We utilize the unique attributes of lower vertebrates or invertebrates to develop simple model systems, such as the isolated flounder tubules, to examine the interaction of xenobiotics with physiological systems. We then test the general applicability of conclusions based on these models by applying them to mammalian test systems. In addition, we try to define each problem at several levels of organization from the intact animal and cell to isolated membrane vesicles. Thus, we are able to more completely evaluate the significance of effects observed at any one level.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Excretion of polycyclic aromatic hydrocarbons (PAH)--PAH are metabolized by the P-450 mixed-function oxidase (mfo) system in marine teleosts. The mfo system is also highly inducible in teleost fish upon exposure to PAH. Analysis of position-specific metabolism has shown that the bulk (50-70%) of PAH metabolism in both control and induced fish takes place on the benzo-ring of benzo(a)pyrene (BP), producing forms closely related to the carcinogenic mammalian metabolites. Since the fish were able to produce these potentially carcinogenic metabolites from PAH, it was vital to assess the mechanisms controlling the excretion of these compounds. We have shown 1) that different early BP metabolites are excreted at very different rates from each other or from BP itself, 2) that excretion is mediated by the organic anion transport system and 3) that anions (e.g. 2,4-dichlorophenoxyacetic acid ([2,4-D]) effectively retard excretion of BP metabolites. Recent emphasis has been on factors which might alter the rate of excretion and provide a mechanistic basis for the widely different (10-fold or more) clearances of individual metabolites. First, we examined the influence of altered P-450 activity on BP metabolite excretion. Neither induction of P-450 by 3-methylcholanthrene pretreatment nor the increase in P-450 activity produced by elevation of ambient water temperatures changed the excretion pattern or the distribution of the metabolite in the flounder. This result suggested that other steps, such as conjugation of the metabolite, rather than P-450 activity *per se* were rate-limiting for the excretion process. HPLC analysis of the form of the BP actually excreted was consistent with this hypothesis. The rapidly excreted metabolite, BP-7,8-diol, was excreted predominantly as its sulfate conjugate (65%), with lesser quantities (30%) as the glucuronide conjugate. On the other hand, BP-7-phenol and other phenolic metabolites (excreted at only one-tenth the rate of 7,8-diol) were excreted predominantly (90-95%) as their glucuronide conjugates, suggesting a greater affinity of the renal organic anion transport system for the sulfate conjugate.

We will approach this problem in three ways. First, we will utilize the isolated renal tubules of the flounder to examine directly the affinities for sulfate and glucuronide conjugates. We will also be able to use this preparation to examine the mechanism responsible for 2,4-D inhibition of BP metabolite excretion previously seen in vivo and to assess quantitatively its sensitivity to 2,4-D and related anionic xenobiotics. Secondly, we will examine the influence of in vivo alterations in conjugation reactions on the excretion of BP metabolites. Finally, we will use isolated lumenal and contralumenal membranes from both teleost and mammalian kidney to characterize the driving forces responsible for organic anion transport and the selectivities of the system at the membrane level.

2. Other projects: We have continued two collaborative projects this year. The first, with Dr. A.L. Krall, deals with the potential for organ specific toxicity secondary to accumulation of the foreign compound via organic anion transport. We have shown both uncoupling of oxidative phosphorylation and inhibition of respiration in mitochondria from liver and kidney after exposure to anionic xenobiotics in vivo and in vitro. Associated with these responses are characteristic inhibition of mitochondria ATPase activities. We are currently examining the effects of these changes in mitochondrial membrane function on Ca^{++} and proton transport and regulation in mitochondria.

The second, with Dr. O'Tauma and colleagues, deals with extention of our previous observations that anion xenobiotics are both substrates and competitive inhibitors of organic anion transport at the choroid plexus. This system is critical for the regulation of concentration of anionic metabolites of neurotransmitters within the brain and cerebrospinal fluid. We have shown in vivo through ventriculo-cisternal perfusion studies that 2,4-D shares the same transport system as the neurotransmitter metabolites and that its elimination from the brain may be retarded by aspirin and its metabolites. We have also shown that these anions will inhibit respiration of the plexus, thus substantially changing all plexus functions. We will examine the influence of aspirin, alone and in conjunction with 2,4-D, on cerebrospinal fluid production and transport rate in vivo and in vitro.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms controlling the ability of marine organisms to eliminate the many foreign compounds reaching the marine environment is vital in predicting the hazards of subsequent consumption of these species by man. Furthermore, the use of model preparations such as the isolated flounder renal tubule permits rapid assessment of the interaction of xenobiotics with renal function, or in the case of organic anions, such as 2,4-D, with other similar transport sites in the body. The confirmation of 2,4-D and DDA inhibition of choroid plexus transport of a normal, but toxic, brain metabolite in the rabbit is an excellent example of the predictive value of such a model system from the marine environment. Finally, the addition of the ability to study membrane transport in vesicle preparations will now allow us to study the details of the interactions of xenobiotics with membrane transport independent of the effects on other cellular organelles.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80037-03 LP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Drug and Xenobiotic Metabolism in the Lungs: Mechanisms and Modifying Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Kenneth G. Jones	Visiting Fellow	LP NIEHS
	James R. Fouts	SES	LP NIEHS
	Theodora R. Devereux	Research Biologist	LP NIEHS
Other:	Kandiah Sivarajah	IPA	LPFT NIEHS
	Thomas E. Eling	Research Chemist	LPFT NIEHS

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Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION
NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the effects of various chemicals on drug and xenobiotic metabolism in various types of cells in the lung. Of particular interest are those compounds which are metabolized by the cytochrome P-450 monooxygenases. Both rats and rabbits are being used as experimental animals. The rat was chosen because it, like man, is subject to induction of these metabolizing systems by certain drugs and chemicals. The rabbit is being used as a model because of the particularly high xenobiotic metabolism in the lungs of this species. Currently, different cell types, especially alveolar type II cells and Clara cells are being isolated so that a detailed examination of the toxication-detoxication processes in purified cell types and in individual cells may be carried out. Different intrinsic abilitities to metabolize various chemical compounds including benzo(a)pyrene, and these activities are inducible in rats to different extents. Also, treatment of rats with monooxygenase inducers alters the balance of toxication-detoxication enzyme systems in the different lung cell types. The development of microspectrofluorometric techniques for the measurement of xenobiotic metabolism has enabled measurements to be made on single isolated cells, rather than on pooled fractions of cells.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various protease solutions are used to digest lungs for preparation of individual cells. Separation of specific cell types is achieved by use of centrifugal elutriation, density gradient centrifugation, and polyethylene glycol-dextran phase separation techniques. Counting and sizing of cells is done with a Particle Data cell counter and channelizer. Enzyme assays are performed using spectrophotometric, fluorometric, and radioisotopic methods. Microspectrofluorometric techniques are being developed for single cell assays on a Zeiss fluorescence microscope equipped with a photomultiplier and monochrometers.

MAJOR FINDINGS AND PROPOSED COURSE: Fractions of rat lung cells enriched either in alveolar type II cells or nonciliated bronchiolar epithelial cells have been prepared. Type II cells have been purified to near homogeneity. Clara cells have been prepared approximately 50% pure. Both types of cells contain monooxygenase activity and metabolize benzo(a)pyrene and 7-ethoxycoumarin, although the intrinsic activity of Clara cells appears to be several times that of alveolar type II cells. Administration of β-naphthoflavone or 3-methylcholanthrene to rats 48 hr prior to sacrifice caused induction of aryl hydrocarbon hydroxylase and 7-ethoxycoumarin in whole lung homogenate and in both the Clara cell-enriched fraction and the alveolar type II cell fraction. Epoxide hydrolase activity has been found in the lung cell digest and purified Clara cells, but was below the level of detection in type II cells. Similarly, glutathione transferase activity was found to be at least twenty times higher in Clara cells than in type II cells. Although both epoxide hydrolase and glutathione transferase activities were not increased by pretreatment of animals with β-naphthoflavone, UDP-glucuronosyltransferase activity, which was present in both Clara cells and type II cells, was found to be increased following pretreatment of rats with β-naphthoflavone.

Arachidonic acid-dependent cooxygenation of benzo(a)pyrene 7,8-diol was found in both alveolar type II cells and Clara cells from rat lungs. In type II cells, the arachidonic acid-dependent oxidation of BP-7,8-diol was greater than the NADPH-dependent rate, while the reverse was found to be true in Clara cells. Both mechanisms were induced by pretreatment of rats with β-naphthoflavone. Also, Clara cells were found to be true in Clara cells. Both mechanisms were induced by pretreatment of rats with β-naphthoflavone. Also, Clara cells were found to metabolize arachidonic only to 6-keto-PGF whereas type II cells produced 6-keto-$PGF_{1\alpha}$ and HHT. This suggests a heterogeneous distribution of the PG-forming enzymes in the different cell types.

Techniques have been developed for the measurement of xenobiotic metabolism in single isolated lung cells by use of a computer-controlled microspectrofluorometer. This technique is being used to compare enzyme activities in different lung cell types from both rabbits and rats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung is a tissue which is in direct contact with the environment and a host of airborne xenobiotic substances, some of which are presumed to be harmful and to cause disease. How the lung (and the individual cells within the lung) handles such xenobiotics may determine whether or not these chemicals are ultimately harmful. This research is directed toward an understanding, in biochemical terms, of how

the various toxication-detoxication processes occur in the lung and in the individual cells of the lung. In addition, an understanding of factors which modify or alter these processes will contribute to a rational basis for assessment of the risks to health posed by various xenobiotics in the environment.

PUBLICATIONS

Fouts, J. R.: Drug Biotransformation in Lungs and Isolated Lung Cells. Czechoslovak Journal of Occupational Medicine (Pracorni Lekarshi) 32, 1980, pp. 355-360.

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LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

THE LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY
Summary Statement

The purpose of the Laboratory of Pulmonary Function and Toxicology (LPFT) is to investigate the biology and pathobiology of the respiratory tract in order to extend our basis for understanding mechanisms underlying various lung injuries and lung diseases. Since it is not possible to cover all aspects of pulmonary function and disease within the scope of one laboratory, LPFT's work is focused on a few research areas which seem important and in need of development. Accordingly, LPFT is divided into six independent research groups, each having its own central research theme. The different groups nevertheless collaborate on many research projects which makes it possible that diverse types of expertise can be brought to bear on a given problem.

I. The cellular complexity of the lung has made investigations of many lung functions difficult. We, therefore, decided that it was important to make a major effort in the development of *in vitro* approaches for studying the function and the regulation of biosynthetic activity of major pulmonary cell types. The work of the Cell Biology Group has been devoted to this goal over the last several years and has been specifically concerned with defining essential parameters for studying growth and differentiation *in vitro* of epithelial cells derived from the conducting airways. This work has progressed to the point where it has now become feasible to study the regulation of mucus biosynthesis as well as differentiation of specific cell types in culture. Such studies have recently been initiated. At the same time, the Cell Biology Group has made major contributions to the development of epithelial transformation systems using rat and hamster tracheal epithelial cells (see below).

II. Only recently has it been recognized that the lung might be an important target organ for a variety of hormones, and that it might itself contain cells with endocrine activity. One of the most intriguing cell types to be found in the conducting airways are the neuro-endocrine cells, which occur either singly, seemingly dispersed at random in the epithelium or in groups, organized as neuro-epithelial bodies. These cells which constitute only a minor cell fraction in the airways (overall approximately 10^{-4}) are recognized by their histochemical and ultrastructural characteristics. Their function in the respiratory tract is unknown. One of the major research efforts of the Endocrinology Group over the last several years has been to try to establish experimental approaches to study this enigmatic cell type, to identify endocrine products associated with them, to study cell turnover rate to isolate them and to grow them in culture. Since they are also thought by many to be the origin of a special type of bronchogenic carcinoma, the oat cell carcinoma, which is commonly associated with ectopic hormone production, their response to the carcinogen, diethylnitsosamine, was studied, since this carcinogen appears to have a special affinity for this cell type.

Another project, which has been pursued by this group is the isolation and characterization of two peptide hormones, namely a physalaemin-like peptide and bombesin. Both physalaemin and bombesin have originally

been described as amphibian hormones. Recently similar peptides have been shown to occur in mammalian tissues including lungs, but their normal physiologic function in mammals is uncertain.

III. Another potentially very important endocrine function of the lung is related to the prostaglandins. Prostaglandins are a group of fatty acids with varied biological activity. Among the known functions are control of cell growth, and regulation of vascular tone. They also play an important role in inflammation and platelet adhesion. The lungs of many species contain high levels of prostaglandin synthetase activity. The biosynthesis, transport and function of these fatty acids are investigated by the Prostaglandin Group. Endothelial cells are particularly rich in PGI_2 synthetase and may actually be the major source of that activity in the lung. Physiologic factors such as respiration rate and various chemicals stimulating or inhibiting production of prostaglandin(s) and thromboxane in the lungs are being studied. Also being examined are species differences in pulmonary prostaglandin synthesis. These studies include human tissues. Recently this work has been extended to an examination of various pulmonary cell types including alveolar type II cells, Clara cells and pulmonary macrophages. Another aspect of this work is concerned with the role of prostaglandin(s) in carcinogenesis and of prostaglandin synthetase in the metabolism of procarcinogens including polycyclic aromatic hydrocarbons aromatic amines and diethylstilbestrol. Close collaboration exists between the Environmental Carcinogenesis and Prostaglandin Groups.

IV. A long-standing interest of ours has been the pathogenesis of respiratory tract cancer particularly the evolution of pre-neoplastic and neoplastic cell populations during the "postinitiation phase." The recent advances in the culture of airway epithelial cells have made it possible for the Environmental Carcinogenesis Group to develop epithelial cell transformation systems. The goal of these investigations is to analyze the qualitative and quantitative cellular changes occurring during neoplastic transformation and to determine the phenotypic alterations characteristic of this cell type, during various phases of neoplastic development. Furthermore, studies are being conducted to investigate major factors modulating the progression of transformation in the epithelial cell system and to study their effects on the rate of progression. Other investigations are concerned with the relationship of carcinogenesis and mutagenesis using cell systems in which both can be studied and quantitated. In this context the role of qualitative and or quantitative chromosomal changes in neoplastic transformation is being investigated. Among other agents the carcinogens, diethylstilbestrol, and asbestos, are being studied. These agents are particularly interesting in this context, since they are typical examples of non-mutagens with well-documented carcinogenic activity in both humans and experimental animals. These investigations are hoped to provide new insights into the mechanism of carcinogenesis.

V. The program of the Pulmonary Pathology Group is concerned primarily with cells and cellular responses in the alveolar region of the airways. This work centers on the effects of asbestos and other fibrous materials on alveolar epithelial cells as well as alveolar marcophages and the early translocation of inhaled fibers in the pulmonary parenchyma. The studies

suggest the proximal alveolar duct bifurcations as a major sight of fiber deposition. The earliest pathologic alterations following asbestos inhalation seem to occur in the same region, suggesting that these bifurcations may be a major target in the development of asbestotic disease. It was further shown that the type I epithelial cells are probably the first cell type in the peripheral airways to take up the asbestos fibers. The involvement of macrophages in clearance and translocation of inhaled fibers is apparently a later event. Other investigations are concerned with the mechanism of intracellular fiber transport. A number of in vitro studies is underway to analyze the early changes induced by asbestos in cell membranes of red blood cells, a classic model for the study of membrane functions, and in various pulmonary cell types. Close collaboration exists between the asbestos studies of the Environmental Carcinogenesis and the Pulmonary Pathology Groups.

VI. The Biochemical aspects of the alveolar lining layer are investigated by the Biochemical Pathology Group. One line of investigation is concerned with the stimulation of acid hydrolase production by the lysosomes of alveolar macrophages. The interest in this problem was stimulated by the serendipitous finding that small molecular weight substances related to morpholine induce hydrolase synthesis. At present, studies on structure-function relationships are being conducted in the hope of finding the chemical principal underlying this biological activity. Studies on the regulation of secretion and removal of surfactant phospholipids from the alveolar parenchyma have been hampered by the fact that it is not possible to clearly separate the extra cellular from the intracellular compartments. Experiments are currently in progress to develop experimental approaches which will make it feasible to arrive at reliable estimates of the distribution of surfactant between extra and intracellular compartments. These methods will then be applied to investigate factors controlling the two surfactant pools and agents disrupting the relevant control mechanisms. A project which has been underway for several years is a study of pulmonary alveolar proteinosis. These investigations have resulted in partial characterization of the soluble and insoluble phases of the materials obtained by lavaging alveolar proteinosis patients. The studies have suggested that part of the insoluble fraction consists of an abnormal form of tubular myelin and is composed of multilamellated phospholipid structures separated by proteins. The nature of these proteins is being investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25001-05 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Mutagenesis in Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
Others:	T. Hesterberg	Postdoctoral Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)
F. Talley, EBCB, NIEHS
J. McLachlan, LRDT, NIEHS

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.50	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to elucidate the cellular and molecular mechanisms of neoplastic development and to understand how environmental agents influence the progression of normal cells to malignancy. The specific aims of the research are: (1) to determine whether carcinogen induced mutations are important in carcinogenesis; (2) to determine the nature of critical carcinogen induced genetic changes (i.e., whether they are gene mutations, chromosomal aberrations or rearrangements, or numerical changes in chromosomes); (3) to understand the mechanism of action of certain environmental carcinogens which are possibly nonelectrophilic (e.g., diethylstilbestrol, asbestos and nickel; and (4) to determine the mechanism by which tumor promoters enhance or induce cell transformation.

PHS-6040
(Rev. 2-81)

METHODS EMPLOYED: Syrian hamster embryo fibroblasts are exposed to various carcinogenic and mutagenic perturbations and induction of morphological and neoplastic transformation, gene mutation, chromosome aberrations and aneuploidy are all measured in same target cells as described by Barrett and Ts'o (Proc. Natl. Acad. Sci., USA, 75, 3297-3301, 1978). The induction of mitotic aneuploidy is also measured in a genetic system employing the yeast Saccaromyces cerevisiae described by Parry et al., Nature 294: 263-265 (1981).

MAJOR FINDINGS AND PROPOSED COURSE: Diethylstilbestrol (DES) is known to be a carcinogen in humans and rodents. However, DES is not active as a mutagen in bacterial mutagenesis tests. Using the Syrian hamster embryo system, we have reported that DES is capable of inducing neoplastic transformation of Syrian hamster embryo (SHE) cells in culture. Under the conditions which result in transformation, DES fails to induce somatic mutation at two genetic loci. This is the first example of a definitive dissociation of mutation and transformation measured in the same cellular system. These results suggest that DES can transform cells through a mechanism other than point mutation, a frameshift mutation, or a small deletion. Of course, other mutagenic changes, which are not detected by these two mutational markers, may still be important. One example of this is a chromosome mutation. DES is known to induce genetic damage in cells by chromosome loss, and a recent report demonstrates a correlation between aneuploidy and vaginal cellular dysplasia in young women exposed prenatally to DES.

We have shown that DES induces numerical but not structural aberrations in SHE cells under conditions which result in DES-induced cell transformation. Four lines of evidence suggest that DES-induced aneuploidy is an important factor in its carcinogenicity: 1) DES-induced neoplastic cell lines are aneuploid; 2) DES induces aneuploidy at concentrations which induce neoplastic transformation; 3) a correlation between induction of aneuploidy and cell transformation exists with DES and its analogs; and 4) experiments with synchronized cultures indicate that DES treatment during mitosis is most effective in inducing cell transformation and aneuploidy.

2-Aminopurine (2-AP) is a classical mutagen in bacteriophage and bacteria, but is not carcinogenic in animals. We have shown that 2-AP induces somatic mutations and morphological transformation of mammalian cells in culture, but its activity is very weak for both endpoints. This may explain its lack of activity in animal carcinogenicity tests. A related compound, 6-N-hydroxylaminopurine (6-HAP) is a much more potent mutagen and in this system induces significant morphological transformation, which correlates with its carcinogenicity in animals. These results indicate that base analogs do have the potential to be carcinogenic which supports a correlation between mutagenicity and carcinogenicity. However, the ability of modified purines to induce neoplastic transformation correlates qualitatively but not quantitatively with their ability to induce gene mutation. On the other hand, their ability to induce chromosome mutations does coincide with their transforming activity.

In collaboration with J. Parry (Swansea), we have shown that TPA induces mitotic aneuploidy but not recombination in yeast. Furthermore, the ability of other phorbol ester derivatives as well as non-phorbol ester promoters to induce aneuploidy in this system correlates with their tumor promoting activity. Mezerein, which is very similar to TPA in inducing phenotypic cellular changes

but is only a weak tumor promoter, is inactive in this aneuploidy assay. Our data support an hypothesis that tumor promoters act by stimulating the expression of recessive mutations (presumably in regulatory genes) produced by an initiating agent or by altering gene balance, both of which could result from changes in chromosome numbers as the result of chromosome aneuploidy. The effects of TPA and other promoters on mammalian cell transformation and aneuploidy are under investigation.

These studies will be continued with other carcinogens, such as asbestos and nickel salts.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fifty to ninety percent of all human cancers are environmentally related. Before effective programs in cancer prevention and control can be formulated, a better understanding is required of the mechanisms by which environmental agents induce and promote neoplasia. Since cancer is primarily a cellular disease, this project utilizes model systems of cells in culture to study the cellular and molecular mechanisms of environmental carcinogenesis. Somatic mutation as the basis for the heritable alteration of malignant cells is a major theory of carcinogenesis. In order to test this theory, we have studied the relationship between mutagenesis and carcinogenesis in a cellular system in which both endpoints can be measured concomitantly. In particular, we have bee studying the effects of compounds which are reported to be exceptions to the correlation between mutagenesis and carcinogenesis, i.e., mutagens that are noncarcinogens (for example, nucleic acid base analogs) or carcinogens that are non-mutagens (for example, diethylstilbestrol). This approach should allow a critical determination of the relationship between mutagenesis and carcinogenesis without the complications that exist in comparing the two processes in vastly different assay systems.

PUBLICATIONS

Barrett, J. C., Wong, A., and McLachlan, J. A.: Diethylstilbestrol induces neoplastic transformation without measureable gene mutation at two loci. Science 212: 1402-1404, 1981.

Barrett, J. C.: Gene mutation and cell transformation of mammalian cells induced by two modified purines, 2-aminopurine and 6-N-hydroxylaminopurine. Proc. Natl. Acad. Sci., U.S.A. 78: 5685-5689, 1981.

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Sisskin, E. E., and Barrett, J. C.: Hyperplasia of syrian hamster epidermis induced by single but not multiple treatments with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Cancer Res. 41, 346-350, 1981.

Sisskin, E. E., Gray, T., and Barrett, J. C.: Correlation of sensitivity to tumor promotion and sustained epidermal hyperplasia of mice and rats treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Carcinogenesis, in press.

Elmore, E., and Barrett, J. C.: Measurement of spontaneous mutation rate at the Na^+/K^+ ATPase locus (Quabain resistance of human fibroblasts using improved growth condition. Mutation Research, in press.

McLachlan, J. A., Wong, A., and Barrett, J. C.: Morphological and neoplastic transformation of Syrian hamster embryo fibroblasts by diethylstilbestrol and its analogs. Cancer Res., in press.

Sheela, S., and Barrett, J. C.: Degradation of basement membrane mediated by cellular plasminogen activator. Carcinogenesis, in press.

Barrett, J. C., Brown, T., and Sisskin, E. E.: Deacylation of 12-O-[^{3}H]-tetradecanoyl-phorbol-13-acetate and [^{3}H] phorbol-12,13-didecanoate by hamster skin and hamster cells in culture. Cancer Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25002-05 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

In Vitro Carcinogenesis and Promotion Studies with Respiratory Tract Epithelium.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. Nettesheim	Chief, LPFT	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
Others:	S. B. Pai	Visiting Fellow	LPFT	NIEHS
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COOPERATING UNITS (if any)

Fred Talley, EBCB, NIEHS

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.25	6.25	2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to understand the cellular and molecular mechanisms of neoplastic progression of respiratory tract epithelial cells. The specific aims of the study are: (1) to define the stages in neoplastic progression of epithelial cells; (2) to determine the role of mutagenesis in neoplastic progression of epithelial cells; (3) to determine the role of tumor promoters in neoplastic progression of epithelial cells; and (4) to determine the effect of carcinogen dose on neoplastic progression of epithelial cells. These studies employ a cell culture model for the quantitation of preneoplastic and neoplastic conversion of rat tracheal epithelial cells following treatment with chemical carcinogens and tumor promoters.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat tracheal epithelial cells are grown in tissue culture medium supplemented with fetal bovine serum, hydrocortisone and insulin on collagen coated dishes with 3T3 conditioned medium or on lethally irradiated 3T3 feeder layers. The cells were exposed to carcinogens and/or tumor promoters and cell survival was determined by colony forming efficiency or total number of viable cells. Carcinogen-induced growth-altered, preneoplastic variants are selected by growth in medium without 3T3 factors (which are required for the growth of the normal cells).

At various times after carcinogen/promoter exposure, the cells were assayed for anchorage independent growth in soft agarose and tumorigenicity in nude mice. DNA measurements were performed on whole fixed cells stained with Hoechst and analyzed by flow cytofluorography. Protein synthetic patterns were analyzed in preneoplastic and neoplastic cells by labeling the proteins with 14-C amino acids, separating them by gel electrophoresis, and visualizing them by autoradiography.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of monolayer cultures of tracheal epithelial cells to MNNG induced growth alterations in the cells which led to the establishment of "immortalized" cell lines. The cell lines were tested for anchorage independent growth at the 20th passage. Cells derived from seventy percent of the cultures receiving two or more exposures to MNNG grew in soft agarose. None of cell lines formed tumors in nude mice at the 5th passage. Tumorigencity at the 20th passage is still incomplete, but so far 3 of the cell lines receiving 6 MNNG exposures have formed tumors. Histological examination of the tumors indicated that squamous cell carcinomas were formed at the inoculation site.

Previously we had shown that tracheal cells exposed to DMBA *in vivo* were altered compared to unexposed control cells in their ability to grow without 3T3 conditioned medium and collagen substratum. These studies have been extended successfully to identify selective conditions for cells altered by carcinogens following *in vitro* exposure. In normal F12 growth medium carcinogen altered cells grew in the form of densely packed epithelial foci (EF). The number of EF which grew per culture was dependent upon carcinogen dose. Increasing calcium concentration in the medium at Day 18 did not enhance the number of EF's measured at Day 30 at each MNNG concentration. Removal of conditioning factors at Day 18 eliminated the dose response and gave a low consistent yield of EF's. Control cultures contained no EF's. Switching the culture medium to a serum-free Waymouths medium from Days 18-30 actually enhanced the dose response yielding about twice the number of EF's at each carcinogen concentration. The cells selected by the various conditions are being tested for tumorigenicity in nude mice to validate the selection process.

Having established selection methods for carcinogen-altered cells, we used these conditions to quantitate the frequency of these changes and the time required after carcinogen treatment for their induction. Using MNNG as a paradigmatic carcinogen, we examined the influence of this chemical on the transformation of RTE cells. We found that the frequency of enhanced growth variants increased with increasing dose of carcinogen from 0.3% per colony forming cells treated with 0.1 μg/ml MNNG

(83% survival) to 3.7% with 1.0 μg/ml MNNG (50% survival). The frequency of cell transformation on a per cell basis was independent of the number of cells treated. The transformation frequency was constant over a range of expression times from 2-14 days. Longer expression times in some experiments resulted in a decreased transformation frequency.

In collaboration with Dr. Reen Wu, LPFT, a hormone supplemented serum-free medium was developed in which both premalignant and malignant cells could grow. There were no growth differences at optimal levels of hormones between nontransformed and transformed. However under reduced insulin and transferrin levels the transformed cells grew 1½ to 2-fold better than nontransformed cells. We have cloned the premalignant and malignant cell populations to reduce variability, tested their tumorigenic potential in nude mice and selected clones which retained the parental properties. We are currently testing the hormonal requirements of these clonal populations. Since the etiology of cancer cells is said by many to involve a defect in cellular differentiation, we have used the same non-transformed/transformed cells as above to examine differences in cellular protein synthesis. It is clear from electrophoretic separations of soluble proteins that at least 9 major band differences exist between transformed tracheal epithelial cells and normal primary cells. Of these 9 differences, only three major band differences separate the non-tumorigenic cell line and the tumorigenic cell line. The most dramatic changes associated with increasing tumorigenicity were a large increase in a 53K protein and the appearance of a 13K protein only in the tumorigenic cell line. Two dimensional polyacrylamide gel analysis showed a wide array of alterations, the most apparent in areas containing structural proteins, such as actin.

Using tracheal primary cell cultures we have found that biweekly one hr. exposures from day 6 to day 30 to various TPA concentrations caused dose dependent alterationas in growth capacity. We have determined concentrations of TPA which produce high and low incidences of cell line induction. Using these basic data, we have developed a quantitative epithelial cell system to study the effects of TPA on initiated cells. We have exposed primary tracheal epithelial cell cultures to low and high concentrations of carcinogen/promoter in a sequential manner and measured in vitro growth using a number of assays. At the lower concentrations of MNNG/TPA, TPA's only differential effect on initiated versus non-initiated cells was observed in terms of increased colony forming efficiency at Day 40. Perhaps either the doses were too low or the endpoints were measured too early after exposure.

At higher concentrations of MNNG/TPA, we saw significant increases in cell number at day 40 of the cultures treated with either MNNG or TPA over control cultures, but TPA exposure following MNNG did not enhance the cell number over that of cultures exposed to MNNG only. However, when these cultures were replated on Day 40, a 7-8 fold enhancement in the number of colony forming cells was seen in the MNNG + TPA exposed cultures compared to the sum of the numbers of colony forming cells in the groups exposed to either agent alone. The subculturability of MNNG + TPA exposed cultures was 78% while for cultures exposed to MNNG alone or TPA alone, it was 41% and 57% respectively. The most dramatic alteration observed in cultures exposed to MNNG + TPA was measured with the anchorage independent growth assay: 54 percent of the cultures were agar positive at day 60 while 0% and 7%, respectively, of the cultures exposed to MNNG only or TPA only were

agar positive. The transition from anchorage dependence to anchorage independence occurred at a much higher frequency in the cultures receiving the higher dose combinations of MNNG + TPA treatments than in the low dose combination of MNNG + TPA treatments. In vivo tumorigenicity studies are not completed at this time. Concurrent with the low dose studies we measured the DNA content of MNNG/TPA exposed cells at days 40 and 60. The DNA content profiles of MNNG and MNNG + TPA exposed cells at day 40 showed the emergence of new seemingly aneuploid cell populations. The DNA content profile of cells became progressively altered over the next three weeks as seen on day 60. Cells exposed to the combination of MNNG/TPA displayed multiple cycling populations with aberrant DNA content values. Growth in agarose when measured at about the same time was still negative. Therefore, the appearance of "aneuploid" cell populations may correlate with the acquisition of subculturability for these cells, but the new cell populations had not yet acquired the capacity for anchorage independent growth. Future studies should reveal the origin and significance of the newly emergying "aneuploid" cell populations (we have not made chromosome counts but are assuming that the grossly abnormal DNA values are probably indicative of aneuploidy).

These findings suggest that TPA induces cellular alterations in in vitro growth at a far greater frequency in initiated cells than non-initiated cells. The emergence of seemingly aneuploid cell populations may be a very early indicator of chromosomal changes in preneoplastic cells and a useful marker for future studies.

Other studies are currently underway to develop approaches for investigating the presumed progression of benign cells to malignant cells and comparing the growth and differentiation of normal, benign and malignant cells in culture. Hamster tracheal cells are employed in these studies. Papillomas are induced with diethylnitrosamine and carcinomas will be induced with methylnitrosourea. Our initial studies with normal hamster tracheal cells have led to some interesting results which point to major differencies between rat and hamster tracheal cells in culture. Rat tracheal epithelial cells rapidly lose their differentiated features in primary cell cultures. Within 2-4 days, no ciliated and only few mucus cells can be recognized. So far we have been unable to prevent this loss of morphological differentiation. In contrast, hamster tracheal cells grown on collagen gels with 3T3 conditioned medium display differentiated features including cilia and membrane bound mucin like granules.

Several studies attempting to grow long-term tracheal epithelial cell cultures from DEN exposed hamsters have failed. Even when hamster tracheal papillomas induced by nitrosamine were used, no long-term cultures could be established. This is in contrast to the growth of DMBA or nitrosamine exposed rat tracheal cells in culture. Whether this points to an important difference in species or carcinogen is under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The epithelial cells lining the respiratory tract represent the largest surface of our body which is in direct contact with our environment. Unfortunately malignant neoplasms of the respiratory tract are usually detected at such an advanced stage that prognosis is usually poor. Our current studies continue to be focused on

identifying both early and late cellular alterations during neoplastic progression. Such changes may lead to the development of useful diagnostic tools for early detection of respiratory tract cancer or possibly a short-term carcinogen screening system with a relevant epithelial cell type.

PUBLICATIONS

Steele, V. E. and Nettesheim, P.: Unstable cellular differentiation in adeno-squamous cell carcinoma. J. Natl. Cancer Inst. 67: 1981.

Nettesheim, P., Pai, S. B., Steele, V. E. and Wu, R.: In vitro studies with normal airway epithelium and during its neoplastic transformtion. Proceedings of "In Vitro Epithelial Cell Differentiation and Neoplasia" Meeting. Sydney, Australia, 1982 (in press).

Steele, V. E. and Pai, S. B.: Chemical carcinogenesis in cultured rat tracheal epithelium. Proceedings of 12th Conference on Environmental Toxicology. 1982 (in press).

Steele, V. E., Topping, D. C. and Pai, S. B.: Tumor promotion studies in rat tracheal epithelium. Environmental Health Perspectives, 1982 (in press).

Barrett, J.C., Gray, T.C., Mass, M.J., and Thomassen, D.G.: A Quantitative, Clonal Assay for Carcinogen-Induced Alterations of Respiratory Epithelial Cells in Culture. In Waters, M. and Sandhu, S. (Eds): Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixture. Plenum Press, N.Y., 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25004-05 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tracheal Epithelial Cells in Culture: Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reen Wu	Research Chemist	LPFT	NIEHS
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	T.C. Lee	Visiting Fellow	LPFT	NIEHS
	J.C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	P. Nettesheim	Chief, LPFT	LPFT	NIEHS
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COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Cell Biology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our research goals are: (1) to determine growth requirements for serial and long-term cultivation of normal tracheal epithelial cells in vitro, (2) to investigate series of events occuring on differentiative features (cilia and mucus granules) of tracheal epithelial cells after plating in culture, (3) to elucidate differentiative properties of cultured tracheal cells and their differentiative potential. In vitro culture conditions have been developed to permit serial and clonal cultures of tracheal epithelial cells from rabbits, rats, hamsters and mice. These cells exhibited 10 to 30 population doublings of in vitro life span, and confluent cultures could be passaged 3 to 5 times. We have observed a rapid loss of cilia and mucus granules of cells in culture. Except hamster cells, new cilia were formed after the confluency of culture. Repopulation studies have shown that cultured cells regained mucociliary functions after plating in denuded tracheal grafts. Furthermore, all cultured cells maintained the synthesis and secretion of mucin-like glycoprotein and they were also able to express squamous epithelial properties, which included stratification, cornification and keratinization. These results suggest that these in vitro culture systems can be used to study cell differentiation of tracheal epithelial cells.

PROJECT DESCRIPTION

METHODS EMPLOYED: Epithelial cells were isolated from trachea after the exposure of lumen to protease solution (0.05 to 0.1%, 4°C, overnight). Growth was assessed by counting cells in a Coulter counter model "B". Epithelial nature was determined by immunofluorescent staining of cultured cells with anti-keratin (human) antibody and by transmission electronmicroscope. Denuded (free epithelium) tracheal grafts were prepared by repeatedly freezing and thawing. ^{3}H-glucosamine and ^{3}H-serine were used to label mucin-like glycoprotein in culture, which is separated from the rest of glycoprotein by a sepharose 4B column chromatography. Keratin proteins were extracted from cell lysates after low slat extraction with SDS and reducing agent. Proteins were separated in a polyacrylamide gel.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Serial cultivation of tracheal epithelial cells.

Previous studies have demonstrated long-term cultures of tracheal epithelial cells from rabbits and rats. Continuous efforts were carried out in mouse and hamster cells. Both types of cells required similar growth condition of rat tracheal epithelial cells. However, continuous multiplication of cells depends on the presence of collagen gels but not the coated thin surface. Epidermal growth factor was found growth stimulatory in hamster tracheal epithelial cells. Both cultures can be passaged several times. Studies are in progress to replace the remaining 1% serum level in the culture medium by hormones and growth factors.

2. Characterization of tracheal epithelial cells in primary culture.

The purpose of this study is to understand various responses of primary tracheal epithelial cells to the developed culture condition, which is important to the connection between results in vivo and those in vitro. This study will lead to the illustration of the in vivo origin of cell types adapted to the culture and changes of differentiative features. Previous studies have shown a rapid loss of cilia and mucus granules in primary rat tracheal cultures. These cultured cells regain their mucociliary function upon plating in denuded tracheal grafts. This result now can be extended to the rest of tracheal epithelial cell culture systems. However, only hamster tracheal cells are able to resynthesize cilia at a later confluent culture. This result was supported by a kinetic study and the analyses at the ultrastructural level. Experiments are needed to determine whether the change of protein synthesis is coincided with the morphological observation of ciliogenesis. Other studies include the use of cell separation techniques and cinematographic approaches to determine the fates of cilia and mucus granules in culture and the newlysynthesized cilia.

3. Synthesis of mucin-like glycoprotein and keratin proteins in tracheal epithelial cell cultures.

Previous studies have shown the synthesis and secretion of mucin-like glycoprotein in rabbit tracheal epithelial cultures. This result can now be

extended to other tracheal cell culture systems. Properties of mucin-like glycoprotein synthesized in various culture systems are similar, such as (1) large molecular weight, (2) sensitive to alkaline-borate hydrolysis, (3) isotope precursors for labeling. Further studies have shown that collagen substrate used in rat tracheal cultures enhanced 2 to 3 fold of synthesis of mucin-like glycoprotein. This result plus later effects of retinoids and Ca^{++} suggests that the synthesis of mucin-like glycoprotein is under the control of the environment. Therefore, the tracheal epithelial cell culture systems are useful for studying mucus cell differentiation.

Equally important to the above result, in vitro tracheal epithelial cells express some squamous cell properties. These include a stratification, cornification, and keratinization. We have further studied the synthesis of keratin proteins in culture. These keratin proteins are not soluble in a low salt solution but are able to be dissolved in the presence of SDS and a reducing agent. Analysis of these proteins in a SDS-polyacrylamide gel shows 8 to 10 protein bands separated from 40 to 60 K daltons. No major protein migrate at 67 K was observed. This property is different from that in epidermal cells. Experiments are in progress to determine the relation of these keratin proteins with the expression of squamous properties.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITITE: With more than 40 different cell types with a diversity of metabolic activity in the lungs, the necessity to use in vitro cell culture systems to study pulmonary epithelial function and environment-related problems is quite appearent: the number of variables to be controlled is markedly reduced.

We have shown that long-term cultures of tracheal epithelial cells can be achieved from four different animals. Similar approaches can be used in human tracheal cultures which is considered difficult. A detailed characterization of cultured cells revealed the loss and regaining cell differentiative function. A similar phenomenon may be existed in vivo when tracheal epithelium is exposed to a toxic environment. Such a change in epithelial properties will affect various functions, such as pulmonary defense mechanisms, drug metabolism and mucociliary function. The synthesis of mucin and keratin proteins in culture suggests that the culture system can be used as a model system to study mucus cell differentiation and keratinization.

PUBLICATIONS

Wu, R. + Smith, D. Continuous multiplication of rabbit tracheal epithelial cells in a defined, hormone-supplemented medium. In Vitro, in press, 1982.

Wu, R., Groelke, J.W., Chang, L.Y., Porter, M.E., Smith, D., and Nettesheim, P. Effects of hormones on the multiplication and differentiation of tracheal epithelial cells in culture. In "Growth of Cells in Hormonally defined media". eds., by Sirbaskun, D., Sato, G.H., and Pardee, A. Cold Spring Harbor, New York, Chapter 55.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25007-04 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Particle Translocation in Various Cell Types and Anatomic Regions of the Lung.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Others:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inhalation of chrysotile asbestos causes neoplastic and fibrotic lung disease. In humans it is known that exposure to asbestos dust in both occupational and environmental settings leads to a slowly progressing, interstitial lung disease. The pulmonary lesions are characterized by diffuse fibrosis which is most prominent at bronchiolar-alveolar junctions. We have established the initial deposition sites and translocation pathways of inhaled asbestos. We have presented an intracellular actin-mediated contractile mechanism by which particles are moved through the alveolar epithelium to the interstitium. In addition, an early interstitial lesion (microcalcification) has been demonstrated within alveolar duct bifurcations where asbestos fibers were deposited initially. Epithelial, macrophage and interstitial cell interactions with asbestos lead to progressive alterations in cell number, volume and production of connective tissue components as determined by ultrastructural morphometric techniques.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: White rats were exposed nose-only in plexiglass cylinders for only one hr. to an aerosol of chrysotile asbestos. This brief exposure period allows the investigation of particle deposition patterns which are not confused by chronic or repeated exposures. Lung fixation was carried out by vascular perfusion through the right ventricle and pulmonary artery so that inhaled particulates would not be displaced from the alveolar surface by fixative flow. The following fixation times after exposure were selected to assess initial particle deposition and subsequent translocation:immediately (i.e. 4 min. ± 25 sec.) after exposure, 5 hrs., 1 and 2 days, 1 week and 1 month. Tissue blocks from varying regions of the lung were prepared for conventional transmission and scanning electron microscopy (SEM). High resolution SEM (i.e. point to point resolution of 100Å) was used to identify small asbestos fibrils at the actual sites of initial deposition on alveolar duct bifurcations. To establish whether or not early changes were taking place in various compartments of the bifurcations, these regions were dissected out and embedded in plastic for thick-sectioning and then subsequently thin-sectioned for ultrastructural morphometry.

Special techniques were used to study the nature of intracellular filaments and inclusions of uncertain elemental content: 1) To add support to our hypothesis that contractile filaments play a role in transporting asbestos fibers, selected tissue blocks from exposed animals were glycerinated and treated with heavy meromyosin. This stabilizes actin-containing microfilaments which then can be studied by conventional TEM. 2) To establish the elemental nature of asbestos-induced intracellular calcifications, interstitial cells with suspected calcium-phosphate inclusions were studied by x-ray energy spectrometry. This technique provides the precise determination of intracellular elemental content *in situ* where the lesions were suspected to have developed initially.

MAJOR FINDINGS AND PROPOSED COURSE: Electron microscopy showed that the majority of chrysotile asbestos fibers which pass through the conducting airways deposits at the bifurcations of alveolar ducts. The farther an alveolar duct bifurcation was from its terminal bronchiole, the less asbestos was observed. Compared to fibers in tissues studied immediately after expsoure, the number of fibers present on alveolar duct surfaces was significantly decreased 5 hr. after cessation of the 1 hr. exposure. Often, fibers were taken up by Type I epithelial cells during the first hour of dusting, and this process continued through the ensuing month in which the animals were studied. As early as 24 hr. after exposure, asbestos had been translocated to the interstitium, and there was an accumulation of macrophages at sites of initial asbestos deposition, i.e., at alveolar duct bifurcations.

Inhaled asbestos fibers taken up by alveolar epithelial cells are translocated to the underlying interstitium by intraepithelial actin-containing microfilaments. Electron microscopic studies of lung tissue showed a clear association between intraepithelial asbestos and thin microfilaments. Heavy-meromyosin stabilized (i.e., actin-containing) microfilaments formed complexes around intraepithelial asbestos fibers. Asbestos fibers added *in vitro* to tracheal

explants and to a cultured epithelial cell line exhibited clear associations with microfilaments. Biochemical measurements showed significantly increased levels of polymerized actin in asbestos treated cells when compared to untreated controls. Contractile intraepithelial filaments could provide the motive mechanism to translocate asbestos particles to the pulmonary interstitium where they may injure macrophages and fibroblasts. Further studies in vitro are onging to clarify this issue.

One month after a 1-hr. exposure to chrysotile asbestos, numerous asbestos fibers have accumulated within the lung interstitium at alveolar duct bifurcations. Many of these interstitial fibers were found in the center of intracellular microcalcifications. The presence of calcifications was proven by X-ray energy spectrometric analysis of the inclusions in situ. Clear X-ray peaks for Ca^{++} and P^{++} were demonstrated. It appears that one month after a 1-hr. exposure, fiber-induced membrane injury in interstitial cells leads to formation of microcalcifications. This could represent the presence of early cell injury in the initial pathogenetic sequence of asbestosis. It is not known when lung injury is initiated in asbestos-exposed individuals. In rats, we propose that the rapid accumulation of interstitial asbestos fibers and the consequent formation of intracellular calcifications are initial steps of lung injury which occur sooner after exposure than was heretofore expected. Mechanisms of intracellular injury and calcium accumulation are ongoing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Asbestos is a highly toxic particulate to which individuals are environmentally and occupationally exposed. Very little information is available on the basic cellular mechanisms which lead to asbestos-induced disease. Such information is essential to providing meaningful treatment of pulmonary disease and employing appropriate preventive measures.

PUBLICATIONS

Brody, A. R., Hill, L. H., Adkins, B., and O'Connor, R. W.: Chrysotile asbestos inhalation in rats: deposition pattern, reaction of alveolar epithelium and pulmonary macrophages. Amer. Rev. Resp. Dis., 123: 670-679, 1981.

Brody, A. R., Soler, P., Basset, F., Haschek, W. M., and Witschi, H.: Epithelial-mesenchymal associations of cells in human pulmonary fibrosis and in BHT-oxygen-induced fibrosis in mice. Exp. Lung Res., 2: 207-220, 1981.

Brody, A. R., Roe, M. W., Evans, J. N., and Davis, G. S.: Quantitation of particle distribution in the lungs of rats exposed to chrysotile asbestos and crystalline silica. In press, Scan. Elect. Microsc., 1982.

Brody, A. R., and Hill, L. H.: Interstitial accumulation of inhaled chrysotile asbestos fibers and consequent formation of microcalcifications. In press, Amer. J. Pathol., 1982.

Brody, A. R. Roe, M. W., Evans, J. N., and Davis, G. S.: Deposition and translocation of inhaled silica in rats: quantification of particle distribution, macrophage participation and function. In press, Lab. Invest., 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25008-04 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Elemental Analysis of Asbestos and Studies of Pulmonary Macrophages From Asbestos-Exposed Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
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	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chrysotile is the most commonly used and most toxic asbestiform mineral. It is hypothesized that the elemental nature of the fibers plays an important role in the mediation of disease consquent to inhalation. It also has been proposed that phagocytosis of asbestos fibers by pulmonary macrophages is an essential step in the pathogenesis of asbestosis. Our earlier studies established the elemental ratio of magnesium to silicon in inhaled fibers. Now we have used this knowledge to determine the percentage of asbestos-containing pulmonary macrophages lavaged from animals after aerosol exposure. In addition, we have learned that subtle, but consistent alterations in macrophage morphology, function and enzyme secretion are present only 48 hrs. after a 1-hr. exposure to chrysotile asbestos. By 48 hrs. after a 1-hr. exposure to asbestos, macrophages have migrated to alveolar duct bifurcations where the fibers were deposited initially and where early asbestos-induced lesions are known to develop. Significant accumulations of macrophages are found on over 90% the bifurcations.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats were exposed to chrysotile asbestos for 1 hour. Additional groups of animals were exposed while held individually in cages for 5 hours. The time-related events of the macrophage response to inhaled asbestos were studied by morphologic techniques in concert with gentle lavage procedures. This approach allowed ultrastructural examination of alveolar duct bifurcations (sites of asbestos deposition) at selected times post-exposure and before or after performing pulmonary lavage. Thus we could establish: 1) when and how many macrophages have migrated to bifurcation surfaces, 2) the percentages of these cells which are removed by lavage procedures, 3) the percentage containing asbestos, 4) the morphologic characteristics of the cells in situ and in vitro, 5) the phagocytic capacity of macrophages lavaged from sham and asbestos-exposed macrophages at selected times post-exposure, and 6) the biochemical nature of certain enzymes produced in vitro after asbestos exposure. Each of these parameters is designed to address the central issue: Does the macrophage play a role in mediating asbestos-induced interstitial lung disease? Studies of macrophages on bifurcation surfaces could demonstrate a relationship between sites of cell accumulation and development of lesions. Understanding the morphologic characteristics and correlated phagocytic potential of asbestos-exposed macrophages may provide new data on the ability of such cells to carry out normal functions at the alveolar level. Characterization of enzymes and other products from cells activated by inhaled dust were studied to provide the information necessary for making a connection between exposed macrophages and the stimulation of other lung cells.

MAJOR FINDINGS AND PROPOSED COURSE: Forty-eight hrs. after a 1-hr. exposure to asbestos, an average of 2.4 macrophages was counted by scanning electron microscopy (SEM) on the surfaces of first alveolar duct bifurcations. In contrast, duct surfaces of sham-exposed animals rarely exhibited a single macrophage. Ninety-one percent of the dust-exposed bifurcation surfaces was occupied by at least one macrophage, with many exhibiting up to 10 macrophages per bifurcation. After bronchoalveolar lavage, 67% of the macrophages which had accumulated were removed from bifurcation surfaces of exposed animals. SEM in concert with X-ray energy spectrometry showed that 35% of the lavaged cells contained asbestos in culture. This percentage rose to over 60% form animals exposed for 5 hrs.

No significant differences were found in cell numbers and viabilities of macrophages recovered from asbestos and sham-exposed animals at 48 hrs., 72 hrs., and 8 days after a 1 hr. exposure. Alterations of asbestos-exposed macrophages were quantified by ultrastructural assessment of surface morphology. The most striking change in asbestos-exposed macrophages was in the significantly decreased percentage of cells with ruffled membranes, i.e. there were more smooth-surfaced macrophages recovered from exposed animals. These smooth cells had a deminished capacity to phagocytize iron beads in vitro compared to ruffled cells (e.g. in three separate experiments ∿88% of smooth-surfaced cells phagocytized 0 beads, while only ∿8% of ruffled cells were not phagocytic). We correlated these findings with the effects of toxic environments and dusts on the surface morphology and functional capacity of macrophages, i.e. an enhanced

percentage of attached pulmonary macrophages lost their ruffled characteristics and exhibited phogocytic impairment following exposures to silica and acidic culture conditions. We intend to prove that exposure to toxic substances alters the organization of microfilament-containing structures such as surface ruffles. We have shown here that in vivo exposure to chrysotile asbestos causes decreased percentages of ruffled macrophages and a correlated phagocytic impairment. We will determine whether or not this finding is significant in regard to understanding asbestos-induced lung disease.

Using biochemical measurements, we found a 50% increase in intra- and extracellular levels of acid phosphatase in the cells of asbestos-exposed animals following a 12 hour culture period. These data correlate with cytochemical findings which show an increased degree of acid phosphatase staining in asbestos-exposed macrophages. This response appeared to be restricted to acid phosphatase since the levels of 2 other representative acid hydrolases were not uniformly enhanced. Extracellular levels of lactate dehydrogenase were also increased in exposed macrophages. These findings indicate that phagocytosis of chrysotile asbestos by pulmonary macrophages in vivo produced a measurable biochemical activation. The significance of this activation in causing macrophage mediated disease will be a major focus in this laboratory.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pulmonary macrophage has been implicated as a mediator of interstitial lung disease. Understanding the macrophage alterations induced by inhaled asbestos is essential to explaining the mechanisms of asbestos-related lung fibrosis.

PUBLICATIONS

Brody, A.R. and DeNee, P.B.: Biological Activity of Inorganic Particles in the Lung. In Straub, C.P. (Ed): Critical Reviews in Environmental Control. SRC Press, Inc., Boca Raton, Fla., pp. 277-299, 1981.

Brody, A.R. and Davis, G.S.,: Alveolar Macrophage Toxicology. In Witschi, H. and Nettesheim, P. (Eds): Mechanisms in Respiratory Toxicology. CRC Press, Inc., Boca Raton., Fla., Vol. II, pp. 3-28, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25009-03 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Membrane Interactions with Chrysotile Asbestos

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Others:	L. H. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chrysotile asbestos causes lysis of red blood cells. It has been proposed that the mechanisms of hemolysis are mediated through interactions between asbestos and cell membrane glycoproteins. Our studies support this concept and the following results are reported: 1) Electron microscopy shows that asbestos fibers distort red blood cells and bind to cell membranes which may become wrapped around the fibers. 2) This reaction is prevented by pretreatment of the cells with neuraminidase. 3) The distribution of lectins which bind to membrane glycoproteins is altered by treating the cells with asbestos. 4) Cell distortion and membrane deformation consequent to asbestos treatment correlate with a clear increase in the ratio of intracellular Na^+:K^+ ions. We have labeled sialoglycoproteins on red cell membranes with a marker visible by electron microscopy (a lectin, wheat germ agglutinin (WGA) - conjugated to gold chloride (Au) spheres). Normally the Au-WGA complexes are distributed evenly on red cell surfaces. Our quantitative data show that the number of Au-WGA markers is significantly decreased on the surfaces of red cells which marked cell distortion and may be a basic mechanism through which chrysotile asbestos exerts its toxic effects upon a variety of cell types.

PROJECT DECRIPTION

METHODS EMPLOYED: Blood was drawn (through cardiac puncture) from white rats into a syringe and diluted 1:10 in a soltuion of .15M NaCl, 5 mM glucose and 5mM KCl. Red cells were cleaned by repeated centrifugation and finally suspended in Tris-buffered saline. To study the surface features of red cells, they were fixed in diluted Karnovsky's fixative and critical point dried for scanning electron microscopy.

Red blood cell membranes were labeled with two lectins, one is an agglutinin from wheat germ (WGA) and the other is from the heamolymph of the horseshoe crab Limulus polyphemus. WGA binds to n-acetyl glucosamine and N-acetyl neuraminic (sialic) acid residues on cell membranes. We conjugated these lectins to gold spheres measuring 20 or 50 nM in diameter. These spheres are readily visible in a high resoltuion SEM, and when conjugated to the lectins, provide a map of selected carbohydrates on appropriately labeled cell membranes. Since asbestos is suspected of rearranging cell membrane components, we studied the distribution of the lectin label before and after asbestos treatment. In addition, neuraminidase treated cells were labeled and/or treated with asbestos to determine whether or not the fibers can bind to sialic acid-depleted membranes. To remove sialic acid from red cell surfaces, 10^6 RBCs were incubated in a solution of .15 M NaCl, .05 M Tris-HCl, .01 $CaCl_2$ (pH 7.0) containing 1 IU of neuraminidase (from V. Cholerae) at 37°C for 1 hr.[2] Cells exposed to asbestos for 5 and 15 min. and unexposed time-matched controls were spread thinly on carbon disks with a wooden stick and plunged into liquid nitrogen. The disks then were transferred to a freeze-drying apparatus for 5 hrs. at -50°C. Individual cells were analyzed by X-ray energy spectrometry (XES) for Na^+ and K^+ content in a JEOL 100 CX STEM. The ratio of the number of Na^+ derived X-rays to K^+ X-rays was calculated through computer programming.

MAJOR FINDINGS AND PROPOSED COURSE: We showed by SEM that over 80% of untreated red cells exhibited a normal biconcave morphology through four hours in tris-buffered saline. Chrysotile asbestos caused distortion and deformation of 75-80% of the cells within 15 min. of treatment. Portions of cell membranes were drawn around asbestos fibers. Pretreatment of RBCs with neuraminidase protected over 75% of the cells from the distorting effects of asbestos, strongly implicating sialic acid in the cell reaction. SEM clearly illustrated that the gold-conjugated WGA and Limulus lectins were evenly distributed across the surfaces of normal red blood cells. Pretreatment of RBCs with asbestos severely altered this distribution pattern on distored cells. The number of labeled sites per unit area of asbestos-reacted red cell surface was reduced to less than 30% of the control level. WGA is known to bind to n-acetyl glucosamine and n-acetyl neuraminic (sialic) acid on cell membranes. Our findings suggest that these membrane carbohydrates are altered in quantity and/or distribution by the deforming effects of chrysotile asbestos. Treating red cells with crystalline silica served as an important control inasmuch as silica-induced hemolysis reportedly is not mediated through interaction with sialoglycoproteins. Our results are consistent with this view in that red cells were labeled evenly with Au-WGA before and after treatment with silica.

The releveance of red cell deformation to the actual events which produce hemolysis is not known. It has been proposed that asbestos-induced redistribution of membrane glycoproteins could lead to alterations of Na^+ and K^+ exchange across cell membranes. We have addressed this issue by determining the intracellular Na^+:K^+ ratios in normal red cells and in asbestos-treated red blood cells which have been distorted. The mean intracellular Na^+:K^+ ratio in normal-appearing untreated cells was 0.56 (range = .30-.85). Cells analyzed from 5 to 15 min. after asbestos treatment exhibited a mean ratio of 1.28 (range = .82-2.57). Over 120 cells were analyzed in three separate experiments. These data suggest that red cells distorted by asbestos rapidly lose the ability to balance Na^+ and K^+ ions with the surrounding medium. In future studies we intend to show that the transport of both Na^+ and K^+/ions is adversely affected by redistribution of cell-surface glycoproteins as discussed above. Anomalies of ion transport are known to result in lysis of red blood cells. Whether or not these mechanisms are operative in pulmonary cells affected by asbestos is the subject of ongoing in vitro studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The cytotoxicity of chrysotile asbestos may relate to the fibrogenic potential of the dust. The structural properties of red cell membranes share many features with those of macrophage membranes. Thus, our current studies on mechanisms of cytotoxicity are centered on red blood cell membranes as a model for future studies on pulmonary macrophages and other cell types which may play a central role in the pathogenesis of asbestosis.

PUBLICATIONS

Brody, A.R. and Hill, L.H.: Interaction of chrysotile asbestos with erythrocyte membranes. In Brown, R.C. et al (Eds): The In Vitro Effects of Mineral Dusts, Academic Press, London, In press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25012-03 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Lung as an Endocrine Organ Controlling Intravascular Thrombosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Eling	Research Chemist	LPFT	NIEHS
Others:	J. Boyd	Technician	LPFT	NIEHS
	Vacant	Biologist	LPFT	NIEHS
	G. Reed	Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)

Dr. L. Marnett, Dr. K. Honn, Wayne State University

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.5	1.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this study is to determine the role of pulmonary metabolism of essential fatty acids, e.g., arachidonic acid in the etiology of intra-arterial thrombosis. This study will determine the factors controlling production of prostaglandins and thromboxanes by pulmonary tissue and vascular endothelium. We have found that respiration rate influences the pulmonary production of PGI_2 and TXA_2. Increasing the respiration rate preferentially stimulates PGI_2 biosynthesis. The presence of platelets or platelet membranes in the vascular bed acts as a stimulator of membrane phospholipase, liberating arachidonic acid and resulting in an increased release of PGI_2 from the lung. Using in vitro systems, we have studied the role of peroxidase in control of PGI_2 biosynthesis. Chemicals that stimulate the peroxidase reduce levels of hydroperoxides that inhibit PGI_2 biosynthesis, resulting in a stimulation of PGI_2 production.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandins and thromboxanes were measured by radioimmunoassay, thin-layer radiochromatography and high performance liquid chromatography, with subsequent liquid scintillation counting. Platelet aggregability was measured by a "platelet-aggregation photospectrometer". The metabolism of essential fatty acids and PG-transport were studied with monolayers of cells in culture or whole-cell homogenates incubated in vitro. Histological tools were used (e.g., electron microscopy, immunohistochemistry) to identify and study metabolic activity of cultured cells (fibroblasts and vascular endothelial cells). The interaction between the pulmonary vascular bed and platelets was studied using the isolated perfused rat, guinea pig and rabbit lung, and pig aortic endothelial cells.

MAJOR FINDINGS AND PROPOSED COURSE: There is evidence in the literature that mechanical stimulation increases the formation of prostaglandins. Respiratory movement can be considered a mechanical stimulation, thus we have examined the effect of respiration rate on the pulmonary secretion of prostaglandins into the circulation. Preliminary experiments with rat lung microsomes suggest that both PGI_2 as well as TXA_2 are produced. The isolated perfused rat lung was used as a model system for the studies. At a respiration rate of 50 cycles/min, PGI_2 and TXA_2 were released into the pulmonary vein in a ratio of 4:1. Inhibition of PG biosynthesis suppresses the secretion. Increasing the rate of respiration to 100 cycles/min increased the release of both PGI_2 and TXA_2. The ratio of PGI_2 to TXA_2 increased to 12:1 indicating preferential stimulation of PGI_2 biosynthesis. Thus, respiratory movement may regulate or influence the release of prostaglandins from the lung.

The presence of particulates also alters the release of PGs into the circulation. Infusion of platelets and platelet aggregates into the isolated perfused rat lung caused a significant increase in the release of both PGI_2 and TXA_2. The ratio of PGI_2 to TXA_2 remained at 5:1. Treatment of the lung with the phospholipase A_2 inhibitor mepracine inhibited the platelet induced release of PGs, suggesting that the increased release was due to stimulation of phospholipiase A_2, liberating arachidonic acid from endogenous stores within the lung. Thus one can assume that platelet aggregates that enter the lung in vivo would stimulate the release of prostaglandins.

We have also investigated a mechanism for controlling PGI_2 biosynthesis. The chemical Bayer 6575 is a potent anti-thrombic and anti-metastatic agent. The bioactivity appears to be mediated by increased PGI_2 synthesis. Preliminary experiments on endothelial cells suggest that Bayer 6575 does not cause the release of arachidonic acid from endogenous stores. Using an in vitro system, Bayer 6576 and a number of other agents; i.e., DES, estrogen, isoproterenol, etc., stimulated the formation of PGI_2 in a dose dependent manner. Bayer 6575 did not alter the conversion of PGH_2 to PGI_2 but did increase the conversion of arachidonic acid to PGH_2. These chemicals are substrates for the hydroperoxidase component of PGS and are cooxidized during the formation of PGs. Bayer 6575 serves as a reducing cofactor, stimulating the conversion of PGG_2 to PGH_2. PGI_2 synthetase is sensitive to peroxides. The addition of Bayer 6575 prevents the inhibiting effect of peroxides on PGI_2 synthesis. We propose that Bayer 6575 stimulates

PGI_2 formation by increasing the formation of PGH_2 and protecting PGI_2 synthetase from peroxides. The hydroperoxidase thus serves as a means of controlling the level of peroxides in cells.

Preliminary results show that Bayer 6575 increased PGI_2 formation by endothelial cells and tumor cells, suggesting that the mechanism operates in intact cells. Thus, PGI_2 biosynthesis may be controlled by PG hydroperoxidase, and greatly influenced by various chemicals including estrogens and various vasodilators.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cerebral and coronary strokes (heart attacks) as a result of intra-arterial thrombosis constitute the major cause of death in this country. Little is known of the causes and the mechanisms that control the formation of intra-arterial thrombosis. Studies suggest a role for platelets in development of pulmonary metastasis. Pretreatment of animals with TXA_2 synthesis inhibitors decreased the number of lung metastases resulting from an injection of B_{16} melanoma cells. Furthermore, treatment with PGI_2 also produced a significant reduction in the number of lung tumors. It has been proposed that tumor cells travel in the blood surrounded by a coating of aggregated platelets. These particles are trapped in the lung, resulting in metastasis. Inhibiting platelet aggregation thus reduces the tumor yield. Pulmonary biosynthesis of PGI_2 thus could also be involved in controlling tumor metastasis to the lung. The lung with its vascular bed and extensive endothelial lining apparently plays a major, yet undetermined role in the control of platelet aggregation and thus thrombus formation. Changes in this endocrine function of pulmonary tissue by exposure to environmental agents may have an impact on the state of mechanisms that control the pulmonary formation of PGI_2, and studying this phenomenon should significantly contribute to our understanding of the ability of the lung to prevent intra-arterial thrombosis.

PUBLICATIONS

Boyd, J. and Eling, T. E.: Prostaglandin release and the interaction of platelets with the pulmonary vasculature of rat and guinea pig. Thrombosis Research 19: 239-248, 1981.

Korbut, R., Boyd, J. and Eling, T. E.: Respiratory movement alters the generation of PGI_2 and TXA_2 in isolated rat lungs. Prostaglandins 21: 491-503, 1981.

Korbut, R., Boyd, J. and Eling, T. E.: PGI_2 and TXA_2 release from isolated rat lungs. Prostaglandins 23: 67-75, 1981.

Marnett, L., Siedlek, P., Ods, R. Honn, K., Warnock, R., Tainer, B. and Eling, T: Stimulation of prostaglandin endoperoxide synthetase and prostacyclin synthetase by the anti-thrombotic and anti-metastatic agent, Nafazatram. J. Biol. Chem., submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25015-02 LPFT

PERIOD COVERED
October 1, 1981 to July 30, 1982

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Cells in Rabbit Fetal Lung: A Model System for In-depth Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dispersed throughout lung airway mucosa are individual and innervated groups of cells with unique morphological, cytochemical and biochemical properties similar to neuroendocrine cells (NE) in the G.I. tract, carotid body, taste bud, thyroid and pituitary. The presence of serotonin, amine precursor uptake and decarboxylation (APUD) enzymes, acetyl cholinesterase, neuron specific enolase and bombesin or at least one or more neuropeptides suggests lung NE cells may have sensory or receptor mechanisms linked with respiratory centers in the CNS. These cells are difficult to locate in adult lung. However, 20 to 30% of all lung tumors are believed to be of NE cell origin possibly in response to environmental influences such as cigarette smoke, ozone, hypoxic conditions, asbestos, halo ethers, and diethylnitrosoamine. It is proposed that these cells play an important role in early lung development since they appear to be the first cells to differentiate and appear to be most numerous during the embryonic and fetal periods. Our objectives are to carry out systematic morphological, cytochemical and biochemical studies of NE cells in the late fetal period, near birth. In order to do so, new systems of analysis need to be developed.

PROJECT DESCRIPTION

In the adult and developing lung, there are single and innervated groups of special airway cells whose histology and cytochemistry classifies them as belonging to the peripherial, Amine Precursor Uptake and Decarboxylation (APUD) endocrine system described by Pearse and coworkers. Cells belonging to the APUD system in various organs (i.e., gut, adrenal, thyroid, pituitary, carotid, lung) have common morphological (dense core vesicles, DCV) and biochemical features (amine precursor uptake, biogenic amines storage in DCV, possible co-residence of one or more neuropeptides in DCV) of both neural and endocrine cell types. These cells in lung are referred to as neuroendocrine (NE) cells. Information about them is particularly intriguing as they are estimated to comprise less than 1% of the adult lung cell population yet it appears to be the NE cells or their stem cell precursor that, in response to certain environmental agents, may become tumorigenic. Twenty to thirty percent of lung tumors are small cell carcinomas which may have their origin in the pulmonary NE cells. It has been proposed that lung NE cells play an important role in lung development. Neuroendocrine cell biogenic amines and/or neuropeptides may act as differentiation signals by modulating the changes in glucocorticoid (cortisone to cortisol) metabolism in developing lung which is known to influence epithelial/mesenchymal interactions in embryonic foregut derivatives. The structural complexities and cellular heterogeneity of the lung makes it difficult to investigate these cells. Neuroendocrine cells appear to be more difficult to locate in adult than developing lung where they appear to be the first cell type to differentiate. Investigators have suggested that both the single lung NE cells and innervated neuroepithelial bodies (NEBs) make up a greater proportion of the airway cells in prenatal lungs. Differences in the distribution of single NE cells and NEBs, as well as innervation of the latter, suggests that NEBs may act as airway chemoreceptors while single NE cells may have a paracrine function. The long term objective is to carry out morphological and biochemical studies of NE cells, especially NEBs, in late gestation lung when airway and parenchynal structures are prepared for gas exchange.

METHODS EMPLOYED:

(1) Developed a lung cell isolation and separation method suitable for the recovery of structurally intact NEBs from near-term fetal rabbit lungs for morphological, biochemical and cell culture studies.

(2) Identified and quantitated serotonin, its precursor 5-hydroxytryptophan (5-HTP) and metabolites, 5-hydroxyindole acetic acid (5-HIAA) in isolated fetal rabbit NEBs.

(3) Demonstrated APUD cellular enzyme mechanisms in isolated lung NEBs in order to determine their metabolic integrity.

(4) Identified, quantitated, and correlated the presence of the neuropeptide, bombesin with isolated NEBs.

(5) Began analysis of receptor binding activity in isolated NEB preparations and receptor activity in tissue sections.

(6) Developed an in vitro organ culture system suited for the maintenance of isolated fetal airway structures with bifurcations.

Prior to NEB isolation experiments, morphological, cytochemical and immunocytochemical studies were made on late gestation rabbit lung in order to examine, in our laboratory, NEB structure and cytochemistry in situ. Since NEBs do not occur randomly in lung sections, it was necessary to prepare serial sections from paraffin blocks to select sections containing NEBs, or in the case of plastic embedded lungs, to cut consecutive 20 μm followed by 60 μm sections from which areas containing NEBs were cut out and reembedded for thin sectioning. Appropriate fixation, embedding and serial sectioning were required for fetal lungs used in the cytochemical and immunocytochemical studies which themselves required adaptation of specific methodologies which had to be later re-adapted for application to cytocentrifuged preparations of isolated, whole fetal lung cells.

In order to facilitate biochemical, physiological and cell culture studies of lung NE cells, we need to develop in vitro methods. Our aim was to concentrate NEBs or groups of NE cells. It was important not to enzymatically-reduce minced fetal lung to a single cell suspension from which we would attempt to recover enriched fractions of single NE cells from the numerous other types of lung cells. The recovery of groups of NE cells by cell separation is not a unique idea; it has been applied successfully to pancreatic islets and groups of neural cells from the CNS. Development of the best enzymatic procedure to recover intact NEBs required fetal lung dissociation trials with various enzymes such as trypsin, pronase E, and collagenase at different concentrations and times. Once we obtained a repeatable yield ($5.2 \times 1.6 \times 10^8$) of viable, single cells and cell clumps from fetal lung (8 gms wet weight), we carried out separation trials using unit gravity sedimentation. This method of cell separation allowed the simultaneous application of large numbers of dissociated lung cells and, by using the appropriate gradient and time, separation of cell clumps from single cells in different fractions. Control and separated cell fractions were analyzed for 5-HT, 5-HIAA, DA, DOPAC and bombesin, all of which are biochemical candidates for lung NE cells. New methods for the simultaneous quantification of biogenic amines and their metabolites by HPLC were recently reported by my collaborator. As further confirmation for the presence of NE cells, we measured the relative ability of control and separated fractions to take up the amine precursor, 5-HTP and produce 5-HT and 5-HIAA. The specificity of the reaction was determined by the L-aromatic amino acid decarboylase inhibitor, benzerazide. Comparative measurements for bombesin in controls and separated fractions were determined by radioimmunoassay. We also measured acetylcholine receptor binding of the ligands ^{3}H-quinuclidinyl benzilate and ^{125}I-α-bungarotoxin in control and separated fraction since lung NE cells contain acetylcholinesterase. We have begun collaborative studies to visualize in 29-day fetal lung tissue section, drug and neurotransmitter binding to opiate, bombesin and acetylcholine receptors.

Earlier, we reported an in vitro organ culture system in which fetal rabbit NEB's were maintained as long as 22 days in organized, 1 mm^3 lung explants. In the same study, 7-day old explants were exposed to pharmacological doses of Ca^{++}-ionophore (A-23187) and reserpine which produced changes in the ultrastructural appearance of NE dense-core vesicles. Explant studies of this type are not practical in that all lung explants did not contain NEBs or single NE

cells and it was necessary to screen large numbers of explants to make few NE cell observations. Recent organ culture studies using isolated airways should make NEBs more readily available for in situ analysis and following various treatments in culture. Intact 29-day fetal rabbit airway controls and 7 day cultures maintained with and without nerve growth factor, glucocorticoids and thyroid hormone in a collagen-fibronectin matrix are currently being evaluated morphometrically. The explants are pulse-labelled with ^{3}H-thymidine. Various sized airways and bifurcations are being examined for NE cells and NEBs and thymidine uptake by these cells. Surrounding epithelial cells will be analyzed for cell types and labeling indices.

MAJOR FINDINGS AND PROPOSED COURSE: NEBs were located in unstained 20 μm and 60 μm section by their osmophilia and three dimensional appearance. NEBs were cut out from thick sections, glued on blank Beem blocks and sectioned for transmission microscopy. We examined 41 NEBs and 8 single NE cells using this method. Corpuscular NEB were composed of several columnar cells organized at their apical surface by a juxtaluminal junctional complex. The specialized surface area exposed to the lumen was approximately 314 μm and covered by microvilli. The base of these corpuscular structures indented into the submucosa. Primary cilia extended from individual cells of NEB, basal bodies were occasionally found in NEB cells. Perinuclear and basally-located neurosecretory granules (DCV) were of at least three types depending on size, shape, and presence of an electron-dense, central core with a clear halo next to the vesicle limiting membrane. It appeared that all cells in a NEB had the same type of granules, but all NEB did not. Synapse formation was occassionally observed between NEB cells and nerve terminals in the form of pre- or post-synaptic membrane thickenings. Cells adjacent to and covering single NE cells or the majority of NEB surfaces were undifferentiated with large amount of glycogen. Ciliated cells were occasionally located in juxtaposition to single and small groups of NE cells. Mucous or Clara cell-type granules were not observed. These studies suggest that NEBs and single NE cells are structurally developed before most other cell types in immature airway epithelium.

Cytochemistry: Fetal NE cells and NEB are argentaffin-positive, argyrophilic and stain for acetylcholinesterase. We concluded they did not stain for PAS and PAS-lead hemotoxylin. Serotonin and dopamine (DA) were demonstrated in NEBs by paraformaldehyde-induced fluorescence. The presence of serotonin was confirmed by the localization of NEBs after staining with specific antibodies to serotonin. The failure of fetal rabbit NEBs to stain with neuron-specific enolase (NSE) antibody is not confirmatory as NSE positive cells were not demonstrated in hindbrain or gut positive control tissues with this antiserum. We conclude 29 day fetal rabbit NEBs are differentiated cytochemically.

Cell isolation and biochemical studies: A method was developed for the isolation from late fetal rabbit lung of NEBs with an intact organoid structure. Collagenase with a high specific activity ($\geq$ 190 μ/mg) was required to repeatedly obtain a viable cell suspension composed of single cells and small cell clumps. The degree of dissociation and morphology of the isolated cells allowed the identification of from one to five intact NEBs by silver staining and formaldehyde induce fluorescence in cytocentrifuge cell preparations made from 10^5 lung cells. Immunostaining of cytocentrifuged cell preparation for localization of serotonin and NSE was difficult possibly due to F_c receptors. Electron

microscopy of isolated NEBs showed preservation of the DCV's and desmosomal connections between NEB cells. Reproducible sedimentation profiles and repeatable recovery of 83% of the cells were obtained by unit gravity separation of 3 x 10^8 lung cells. Cell clumps with a greater sedimentation velocity separated from the mode of cells composed of single cells and dimers. Ten percent of the total cells, 98-99 percent of which were in clumps of four cells or more were in fractions which, when analyzed morphologically and biochemically, compared to unseparated control cells and the mode fractions composed of single cells, contained the NEBs and their biochemical and metabolic components. The number of stainable NEBs in cytocentrifuged cell preparation from these fractions varied from one to 20. Although we estimated recovery of as many as 240 NEBs in 2 x 10^6 cells which represented a 2-12 fold increase in NEBs compared to the number visualized in control cytocentrifuge preparations, we realize the enzymatic treatment of NEBs may prevent them from staining with silver or exhibiting fluorescence, thereby making it unreasonable to calculate absolute numbers. The preferential distribution, however, of serotonin and its metabolite, 5-HIAA and intact APUD enzyme systems, as measured by HPLC analysis, in the separated fractions containing the clumps appeared to substantiate NEB enrichment. DA and its metabolites (DOPAC and HVA) were also present in mixed control cells and demonstrated preferential distribution in the separated fractions. Bombesin-like immunoreactivity was also demonstrated in the fraction of fetal lung cells which had the most serotonin, 5-HIAA and APUD activity. Bombesin reactivity was not found in samples of control, mixed cells or in the mode fractions; this provided further biochemical evidence for the separation and enrichment of NEBs. VIP and calcitonin immunoreactivity was not found in control cells or separated fractions. Muscarinic, cholinergic receptor binding activity also appeared to be more selective for the cell fractions outside of the mode. This may be the first evidence for acetylcholine receptors in NEBs. It appears that isolated NEBs retain some of their structural integrity and certain biochemical and metabolic properties similar to their counterparts in situ. These in vitro studies suggest that NEBs in immature airway epithelium are functionally developed.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The structural complexities of whole lung make it difficult to investigate non-respiratory functions of various pulmonary cells, particularly those which occur in relatively low numbers in the majority of smaller airways. Our specific aim is to develop in vitro model systems starting with whole lung and isolated airways. The importance of studies of this nature lies in the fact that neuro-endocrine may be a target for several environmental factors which may be the cause of pulmonary injury and disease. Yet, our understanding of the function and life cycle of these cells is still very rudimentary.

PROPOSED COURSE: Terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25016-02 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Isolation and Pharmacological Mode of Action of the Mammalian Physalaemin-like Peptide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G.D. Jahnke	Chemist	LPFT	NIEHS
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COOPERATING UNITS (if any)

Don Harvan, LEC, NIEHS; V. Erspamer, University of Rome, Italy; H. Yajima, University Kyoto, Japan

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0		

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physalaemin-like peptide (PSLI) extracted from the mammalian gastrintestinal tract was also found in trachea of several species by radioimmunoaasay and molecular sieving chromatography. A partial purification scheme has been developed for rabbit stomach which utilizes lyophilized tissue, extraction in boiling ion formic acid, filtration or centrifugation, ethanol solbuilization and concentration, G-25 or P-4 chromatography, and LH-20 chromatography. The phase using immunoaffinity chromatography is under development. Recovery at each step varies from 70-95%, however, on reverse-phase HPLC, that value is 5-10%. Linear gradients on HPLC resolved PSLI from the amphibian peptide physalaemin which has a considerably longer retention time. Preliminary physiological experiments with HPLC-purified PSLI using the isolated guinea-pig ileum assay demonstrated a contractile response that appeared less than that of the amphibian peptide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lyophilized rabbit stomachs (50 g lots) were pulverized, extracted in boiling 1.0 N formic acid-EDTA-DTT, and clarified. The yellowish solution was extracted with 90% ethanol overnight at 4°C and filtered. The concentrated filtrates were applied to a Sephadex G-25 column (180 x 4.9 cm) or Bio-Gel P-4 (96 x 1.5 cm) and the immunoreactive region pooled and concentrated. This brown material was chromatographed on a Sephadex LH-20 column (96 x 1.5 cm) in iso-propanol:0.1 N formic acid (1:9 by vol). Reverse-phase HPLC used mobile phases containing 0.01 N HFBA or 0.02 M NH_4-acetate with linear gradients from 0 to 80% CH_3CN.

Pharmacological experimentation employed a guinea-pig ileum in Tyrode's solution in line with a Grass polygraph. The contractile response was standardized with both histamine and physalaemin.

MAJOR FINDINGS AND PROPOSED COURSE: The purification scheme development resulted in minimal losses of immunoreactivity: recovery ranged from approximately 70-95%. The first chromatographic step removed 90% of the extraneous ultraviolet absorbing material and the second one eliminated another 85%. This material, <<1% of original solubilized fraction, however, still has a yellow color, indicative of trace contaminants.

Reverse-phase HPLC clearly separated PSLI from physalaemin, however, the recovery on analytical or preparative runs was 5-10%. Lyophilization also caused extensive losses of immunoreactivity. These large losses of PSLI were also encountered on the use of ion-exchange resins, none of which bound the immunoreactivity nor removed any appreciable amount of contaminants. Similarly, chromatography on P-2 columns failed to yield any advantage.

High voltage paper electrophoresis at pH values from 1.7 to 7.9 provided evidence that PSLI is anionic in nature: this was further collaborated by isoelectric focusing which gave a pI of less than 3.5, whereas physalaemin was closed to 4.

Preliminary pharmacological experiments using the guinea-pig ileum assay indicated that HPLC-purified PSLI contracted the muscle, although the response was weaker than physalaemin.

The proposed course of research entails the purification, sequencing and pharmacological mode of action of this new peptide from mammalian tissue. The preparation of an immunoaffinity column for the final stages in its isolation will be developed from one of the 3 antisera prepared against physalaemin. Once the appropriate antiserum has been determined, the IgG fraction will be obtained by protein A chromatography and then couplied to CNBr-activated Sepharose. The conditions of binding to and eluting from the affinity column must be examined. Sequencing will utilize the fast atom bombardment attachment to existing mass spectrographs at the Institute; this allows one to use 50-100 ng of pure peptide, a level within the realm of reality. Once the amino acid sequence is known, the peptide and specific analogs will be synthesized by Dr. H. Yajima (Japan).

Although we plan to carry out numerous pharmacological studies on its mode of action, Dr. V. Erspamer (Italy) has offered to collaborate with us in these bioassays: it is necessary to employ not only different tissues but also the same tissue from different species in addition to live animals. These assays will include, for example, use of an infusion bath, an Ussing chamber, nerve potentials, blood pressure and animal behavior; they measure muscle contractility (ileum, tracheal rings), ion and water flux through epithelium, nerve transmission, and bodily functions. Furthermore, its effects on enzyme systems and cyclic nucleotide levels must be assessed. The synergistic or antagonistic effect on other peptide hormones will be investigated along with any potential modification of the neuroendocrine and mucin-secreting cells of the lung.

Antiserum will be prepared against this peptide in order to conduct immunohistochemical studies in fixed tissue samples. Injection of purified antiserum into pregnant animals, fetuses or neonates may enable us to learn if this peptide is involved in a developmental event.

Receptor analyses will be carried out in order to determine which tissues contain the endogenous receptor; i.e., target tissues for the peptide. These studies lead to studies on the internalization and metabolism of peptides, as well.

SINGIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The discovery of a new neuropeptide that also exists in lung tissue represents a unique set of circumstances. The number of known peptide hormones that currently exists in lungs stands at 5. For the most part, their pulmonary functions remain unknown, but it is not unconceivable that they could function in the regulation of numerous physiological parameters. In particular, PSLI could be involved in ion secretion and water balance - in analogy to physalaemin - which may yield clues to cystic fibrosis at the molecular level. Several other clinically-defined pulmonary syndromes involving constriction of the airways or mucous production may be affected by an imbalance in peptide hormones. The pharmacological and toxicological events that affect pulmonary function in both normal and in disease states must now include a study of the modulatory action of peptide hormones.

PUBLICATIONS

Lazarus, L. H., DiAugustine, R. P. and Soldato, C. M.: A substance with immunoreactivity to the peptide physalaemin in mammalian respiratory tissue. Exper. Lung Res., in press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25018-01 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Synthesis and Secretion of Mucus Glyproproteins by Guinea-Pig Upper Airways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study is proposed to examine the synthesis and secretion of mucus glyproproteins in different regions of the upper airways of the guinea pig. The responsiveness to selected pharmacological agents and neuropeptides will also be compared. The guinea-pig offers an experimental system whereby mucus secretion by the epithelium can be compared in regions devoid or enriched with submucosal glands.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Gel filtration and ion-exchange column chromatography; liquid scintillation counting; gas-liquid or paper chromatographic identification of sugars.

MAJOR FINDINGS AND PROPOSED COURSE: Our efforts so far, to obtain fresh porcine trachea at NIEHS for this study have been severely delayed. Alternately, our earlier inspection of the upper airwyas of the guinea pig revealed a concentration of submucosal glands in the lower larynx and cranial aspect of the trachea. The lower half of the trachea was essentially devoid of submucosal glands. The proportion of ciliated cells and mucous cells appeared constant in the cricoid, tracheal and bronchial nonglandular epithelium. Thus, we considered that this may represent an appropriate system to compare the biochemistry, rates of secretion, and pharmacology of glandular vs. non-glandular epithelia. Unlike other mammalian systems previously examined, this one will enable us to differentiate the cellular origin of the biosynthetically-labeled macromolecules.

Previously published methods will be used to obtain equilibrium labeling of airway strips in vitro in medium 199 using D-[^{3}H]glucosamine (~20 Ci/mmol) and $Na_2{}^{35}SO_4$ (~500 mCi/mmol). Labeled glycoproteins in the medium will be separated from free sugars by chromatography on Bio-Gel A-5 m equilibrated with sodium dodecyl sulfate (0.1%), Tris-HCl (10 mM), and dithiothreitol (0.1 mM).

The comparative responsiveness of the different regions of the airways will be examined with cholinergic, adrenergic, and histaminergic agonists. We shall also study the regional effects of selected neuropeptides such as vasoactive intestinal peptide, which was previously reported to be in autonomic fibers in the submucosal glands.

Our system will also allow us to examine immunologic stimuli. Guinea pigs will be sensitized with ovalbumin and the airway strips exposed to the antigen to determine the regional effects on mucus synthesis and secretion. Mucus secretion is thought to contribute in a variable degree to the bronchial obstruction occurring in asthmatic attacks. If the antigen challenge in vitro produces a significant effect on mucus secretion when compared to nonsensitized airways, selected pharmacological antagonists will be used to identify the mediator.

SIGNIFICANCE TO BIOEMDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The secretion of mucus by the airways is an essential function for clearance and protection. The hypersecretion of mucus is a characteristic of respiratory diseases such as chronic bronchitis, cystic fibrosis, and asthma, and is considered to contribute to the morbidity of a large segment of the population. The relative role and regulation of airway goblet cell and submucosal gland secretions is still not clearly understoood. Systematic investigation of these phenomena may lead to findings that may aid diagnosis or permit clinical intervention of human respiratory diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25019-01 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Characterization of Amphibian Peptides in Human Lung Small-Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. H. Lazarus	Research Chemist	LPFT	NIEHS
Others:	R. P. DiAugustine	Research Chemist	LPFT	NIEHS
	G. D. Jahnke	Chemist	LPFT	NIEHS
	O. Hernandez	Chemist	LEC	NIEHS

COOPERATING UNITS (if any)
Don Harvan, LEC, NIEHS; R. Ilona Linnoila, NCI, NIH; H. Yajima, University of Kyoto, Japan

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We demonstrated the existence of immunoreactivity to 2 amphibian peptides, bombesin and physalaemin, in extracts of a human lung small-cell carcinoma which was propagated in nude (athymic) mice. These immunoreactive peptides were characterized by reverse-phase HPLC coupled to various chemoselective and enzymic modifications. In the presence of a hydrophilic ion-pairing reagent, the rention time of both peptides were reduced by about 2.5 min relative to pH 4.2. At pH 7.0, only bombesin was more strongly retained to the hydrophobic column due to the loss of charge on His. Pyro-glutamate aminopeptidase also produced a bombesin molecule with a higher retention time whereas physalaemin immunoreactivity was lost, confirming the NH_2-terminal specificity of the antibody. Trypsin cleaved both tumor peptides producing fragments similar to the standards. Selective oxidation by oxone confirmed the presence of a sulfur-containing residue (Met).

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(Rev. 2-81)

of the tumor peptides with the synthetic amphibian standards. Furthermore, the study on the molecular biology of the synthesis, processing and regulation of these peptides in vitro will be initiated using both tumor and lung tissue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The presence of peptides in human SCC which appear to be homologous to those existing in frog skin are of vital interest in any study on the physiology of lung function and the induction of lung tumors. We must ask the question whether these amphibian peptides exist in normal tissue or are unique to this neoplasm? And if indeed these tumors have the capacity to produce peptides unchanged in over 300 million years of evolution does lung tissue harbor a specific, but unexpressed genome that regulates their synthesis? It is currently realized that the biochemistry of mammalian tissues and many of its modulatory mechanisms are established in lower organisms and similar peptide hormones, in particular, exist with little or no modification throughout all forms of life. Thus, the assessment of the role of these particular peptides offer the investigator the opportunity of unraveling fundamental pharmacological questions.

PUBLICATIONS

Erisman, M.D., Linnoila, R.I., Hernandez, O., DiAugustine, R.P. and Lazarus, L.H.: Human lung small-cell carcinoma contains bombesin. Proc. Natl. Acad. Sci. 79: 2379-2383, 1982.

Lazarus, L.H., Jahnke, G.D. and Hernandez, O.: Chemical identity of bombesin in small-cell carcinoma: Homology with the amphibian tetradecapeptide. J. Biol. Chem., submitted, 1982.

Lazarus, L.H., DiAugustine, R.P. and Jahnke, G.D.: Human Lung Small-Cell Carcinoma: The Amphibian Peptide Connection. In Becker, K.L. and Gazdar, A.F. (Eds.): The Endocrine Lung in Health and Disease. Georgetown University Press, 1982.

Lazarus, L.H., DiAugustine, R.P., Jahnke, G.D. and Hernandez, O.: Physalaemin: An amphibian tachykinin peptide in human lung small-cell carcinoma. Science, submitted, 1982.

PROJECT DESCRIPTION

METHODS EMPLOYED: Human lung small-cell carcinoma (SCC) was propagated in nude (athymic) mice, boiled in water, cooled, extracted in formic acid and the clarified material lyophilized. The radioimmunoassay (RIA) for bombesin recognized the mid-region portion of the peptide and that for physalaemin, the NH_2-terminal region including the NH_2-terminus. Mutual cross-reactivity was negligible (<0.0001%).

Immunohistochemistry (IHC) used the improved immuno-globulin-enzyme bridge method on vapor-fixed tissues using diethylpyrocarbonate or parabenzoquinone. Parafin-embedded tissues were serial, sectioned, and mounted on slides. Antiserum were diluted 1:1,000 and controls included prior incubation of antisera with excess peptide.

Molecular sieving chromatography used Sephadex G-75 and Bio-Gel P-4 equilibrated in dilute acid. HPLC used a LDC system fitted with a Rhcodyne injector and a Spherisorb ODS 5 μ column. Solvent systems included 0.01 M HFBA, 0.02 M acetate, pH 4.2 and 0.0 2M phosphate, pH 7.0 with linear gradients from 0 to 60 or 80% CH_3CN.

Chemical treatment of tumor extracts and peptide standards included oxone oxidation, and trypsin and pyro-glutamate aminopeptidase digestions.

MAJOR FINDINGS AND PROPOSED COURSE: Immunoreactivity to 2 distinct amphibian peptides, bombesin and physalaemin, were identified in extracts of SCC by RIA and in fixed tissue sections by IHC. The latter method demonstrated the presence of bombesin and physalaemin in 9-14% of the tumor cells: both peptides appeared to be localized in the same cell or closely associated group of cells, but distinct from ACTH containing cells. Gel permeation chromatography revealed immunoreactivity that eluted at the position of the standard: no larger molecular weight forms were detected.

RIA coupled to the resolving power of HPLC and by the use of chemoselective agents yielded the following results for SCC extracts and standard peptides: (1) identical retention times ($\pm$0.1 min, S.D.) at pH 2.3, 4.2 and 7.0; (2) HFBA produced reduced binding of both peptides to the hydrophobic column; (3) use of pH 7.0 to neutralize the charged His residue on bombesin increased retention time by about 20%; (4) oxone treatment oxidized both peptides with a concomitant decrease in the reduced forms of the peptide; the oxidized forms have lower retention times; (5) pyro-glutamate aminopeptidase produced a more hydrophobic bombesin molecular while virtually destroying the physalaemin immunoreactivity (99.8); and (6) trypsin cleavage yielded new immunoreactive peaks with both bombesin and physalaemin corresponding to bombesin (4-14) and physalaemin (1-6).

The future direction of this project lies in the establishment of a realiable RIA for determining the levels of these peptides in the serum of patients with lung tumors. In addition to SCC, other known lung neoplasms must be analyzed for these two peptides, since bombesin has been observed in several lung cancers. The chemical characterization of the sequence by FAB-mass spectrograph will, of course, verify and confirm all the current evidence on the structural homology

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30085-05 LPFT

PERIOD COVERED
October 1, 1981 to September 20, 1982

TITLE OF PROJECT (80 characters or less)
Induction of Macrophageal Lysosomal Enzymes By Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Tombropoulos	Research Chemist	LPFT	NIEHS
Others:	W. Gibson	Chemist	LPFT	NIEHS
	G. E. R. Hook	Research Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Caroline 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms involved in the regulation of production and release of alveolar macrophage lysosomal enzymes are important to inhalation toxicology because of their participation in the digestion of endocytosed foreign materials and their role in the induction of lung damage during their release.

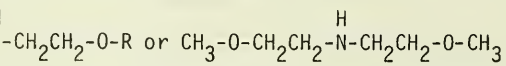
The induction of lysosomal enzymes by morpholine in alveolar macrophages was examined. Morpholine and several related chemicals stimulated the synthesis of α-mannosidase in alveolar macrophages.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: New Zealand white rabbits were used in these studies. For the in vivo experiments, one hundred and six liter capacity stainless steel and plexiglass chambers were used for the exposure of rabbits to 250 ppm of morpholine. Morpholine in the gaseous state was generated by direct evaporation using compressed air (of breathing quality) as the carrier gas. Lung lavages were obtained using antiseptic techniques. The lavages were centrifuged at 600 g for 10 minutes and the lung macrophages isolated. In the in vitro experiments, alveolar macrophages were resuspended in the incubation medium (Medium 199 with Hank's salts 3) mg penicillin and 50 mg streptomycin). The final cell concentration was 1.5×10^6 cells/ml. Five milliliters of the suspension was incubated in 25 ml culture flasks. Three hours after the initial incubation, the medium was poured off, and new medium was added containing the test toxic chemical.

MAJOR FINDINGS AND PROPOSED COURSE: In vivo α-Mannosidase levels in alveolar macrophages were doubled following exposure to morpholine vapors. Other lysosomal enzymes such as acid phosphatase and α-N-acetylglucosaminidase were marginally increased by morpholine (about 130%). Similar results were obtained under in vitro conditions in which isolated alveolar macrophages were cultured in the presence of morpholine. Several compounds related to morpholine such as piperazine and Bis-(beta-methoxyethyl) amine were also found to stimulate α-mannosidase in alveolar macrophages. The induction of α-mannosidase by morpholine and the other inducers were dose dependent. The optimal concentrations for maximal induction varied with the inducer. The induction time was similar for all the inducers tested, but there was a sex difference. The induction of α-mannosidase in macrophages derived from male animals reached its maximum at the fourth hour, whereas, macrophages derived from female animals reached its maximum at the eighth hour of incubation. The chemical structural requirements for the induction of α-mannosidase are 1). The presence of:



2) At least one of the heteratoms must have the basicity of Nitrogen.

3) Oxygen must be of the ether type.

4) Substitutions that increase the basicity have a more pronounced effect on the induction.

Compounds such as dioxane, piperidine, thioxane, ethanolamine, ethylene glycol, 2-(2-Aminoethoxy) ethanol, pyrazine, and tetrahydropyran, which do not meet the above requirements do not induce α-mannosidase activity. These studies will be directed towards the mechanism through which stimulation of α-mannosidase occurs in alveolar macrophages. Initially, studies will be directed towards the identification of receptors for morpholine and its analogs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Lung macrophages provide an early pulmonary defense mechanism. The major defense is based on the ability of alveolar macrophages to endocytose extracellular material and to digest it through their lysosomal enzymes. These same enzymes used for digestion can be released under the influence of certain substances. The release of hydrolytic enzymes can account for extensive tissue damage during acute or chronic inflammation. Therefore, the mechanisms which regulate lysosomal enzyme production and release from alveolar macrophages are important to lung defense and disease studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80008-08 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biosynthesis, Release, Transport and Metabolism of Prostaglandins (PGs) and Hydroxy-Fatty Acids (HFA) in the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Research Chemist	LPFT	NIEHS
Others:	R. Mason	Research Chemist	LEB	NIEHS
	K. Sivarajah	IPA	LPFT	NIEHS
	R. Wu	Research Chemist	LPFT	NIEHS
	J. Fouts	Senior Scientist	LP	NIEHS
	J. Shirley	Technician	LPFT	NIEHS

COOPERATING UNITS (if any)

University of North Carolina; Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.95	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to study the biosynthesis and inactivation of prostaglandins by the lung and other tissues. The effects of environmental agents on pulmonary transport and metabolism of prostaglandins are being investigated, and these effects are being related to pulmonary toxicity and damage. We have compared the formation of PGs from AA and PGH_2 by pulmonary tissue obtained from humans and animals. We have also studied PG formation in rats and rabbit isolated type II, Clara, and trachea epithelial cells. Clara cells made exclusively PGI_2, while type II made PGE_2 and smaller amounts of $PGF_{2\alpha}$ and PGI_2. Trachea epithelial cells did not make PGs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG) thromboxane (TX) and hydroxy-fatty acid (HFA) synthetase activity was measured in vitro using the microsomal protein from a variety of tissues and organs as an enzyme source. ^{14}C-arachidonic acid (AA) or prostaglandin endoperoxides were incubated at 37°C for various times and under several conditions. After incubation, the PGs and TX were removed by solvent extraction, separated by thin-layer chromatography or high pressure liquid chromatography, and estimated by liquid scintillation techniques.

PG biosynthesis was studied by adding labelled arachidonic acid to sonciated cells isolated from rat and rabbit lung. PGs were separated by HPLC. In some experiments cells were maintained in culture for various lengths of times, trypsinized, counted, sonicated, and PGs analyzed. PG biosynthesis was also studied using microsomal fractions.

An isolated perfused rat, guinea pig, or rabbit lung was used to examine the uptake, metabolism, and efflux of prostaglandin (PGs) and their metabolites from lung tissue. The isolated persued lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: Porcine lung microsomes were used to optimize PG biosynthesis. After establishing optimal assay conditions, we compared PG biosynthesis by the microsomal fraction of lung tissue obtained from pig, beef, rat, mouse, guinea pig, and human. Guinea pig lung made large amounts of TXB_2 and very little PGI_2 in a ratio of 15 to 1. With all other species, the ratio of TXB_2 to PGI_2 was 1:1. Human, rat, bovine, and pig lung produce similar PGs.[2] Mouse lung was unique in that PGE_2 was the major prostaglandin biosynthesized. Total prostaglandin biosynthesis activity was very similar in all species studied. Biosynthesis was also studied in cells isolated from rat and rabbit. Rat and rabbit Clara and type II cells were isolated by enzymatic digestion followed by centrifugal elution (in collaboration with Dr. Fouts). Rat Clara cells were about 40% pure while type II were about 80% pure as determined by staining techniques. Rabbit Clara cells were 50% pure while rabbit type II were 80-90% pure. Rat and rabbit tracheal epithelial cells (Dr. Wu) were prepared by enzymatic treatment of the trachea.

Rat Clara cells made exclusively PGI_2 while rat Type II cells made PGI_2 and $PGF_{2\alpha}$. Rabbit Clara cells made PGE_2, with smaller amounts of $PGF_{2\alpha}$. Rabbit type II cells made PGE_2 and PGI_2. In all cases rabbit PG biosynthesis is lower than rat PG biosynthesis. Neither rat nor rabbit trachea epithelial cells made PGs but they did produce high amounts of unidentified hydroxy-fatty acids. Trachea epithelial cells but not Clara or type II cells can be maintained in cultures. We examined arachidonic acid metabolism in cells maintained in culture for 9 days. Surprisingly, the cultured cells produces PGs. Rat tracheal cultured cells produced PGE_2 while rabbit cells in culture made both TXA_2 and PGI_2. The time development of PG biosynthesis was studied in rat tracheal cells. Highest PG activity was observed at 10 days in culture. Further studies are planned with epithelial cells to understand the phenomenon since with other cells, PG activity decreases with

time in culture.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs and HFA have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes. The lung is an important site for the synthesis and metabolism of PGs, alterations in PG biosynthesis, release, transport, and metabolic systems may be related to toxic effects of exposures to pollutants and induction of lung diseases. The lung makes a variety of PGs and HFA but little is known of the particular cells responsible for biosynthesis. This information appears to be important for the elucidation of the role of PGs in pulmonary disease.

PUBLICATIONS

Eling, T., Tainer, B., Ally, A. and Warnock, R.: Separation of arachidonic acid metabolites by HPLC. Methods in Enzymology (in press) 1982.

Eling, T. and Ally, A.: Pulmonary biosynthesis and metabolism of prostaglandins and related substances. Clinical Resp. Phys. 128, 611-625, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80029-06 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Investigations of Human Pulmonary Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Research Chemist	LPFT	NIEHS
Others:	L. B. Gilmore	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

A. Spock, M.D.
Department of Pediatric, Duke University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Identification and characterization of disease-related pulmonary components could provide information concerning the disease process and also function as markers for the diagnosis and monitoring of the disease. Current attention has focused on the multilamellated myelin-like structures present in the alveoli and airways of patients with pulmonary alveolar proteinosis. The objectives of this study have been to elucidate the composition, structure and origins of this unusual material.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Bronchoalveolar lavage effluents from patients with pulmonary alveolar proteinosis were supplied by the Department of Pediatrics at Duke University Medical Center. These lavage effluents were obtained as a by-product of the therapy essential to the well being of the patients.

Insoluble materials were sedimented by centrifugation and then separated from the soluble phase for examination under the electron microscope following dehydration and embedding.

MAJOR FINDINGS AND PROPOSED COURSE: The insoluble particulate materials present in the lungs of patients with pulmonary alveolar proteinosis have been catalogued according to ultrastructural features and quantitated using morphometric analytical procedures. The multilamellated structures are the major component accounting for 42 ± 12% (n = 5 patients) of the total volume of particulate material. Membranous vesicles accounted for 20 ± 7% and aggregates of amorphous material 25 ± 4% of the material. Structures found in the lungs of normal humans, such as tubular myelin and secreted lamellar bodies were minor constituents accounting for less than 2% of the total material.

Structures will be isolated and characterized according to their composition and physical properties.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases except in the advanced states and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed.

Our studies indicate that the major component of the insoluble materials which accumulate in the lungs of patients is the multilamellated structures suggesting that the processes through which the structures are formed may be a major factor in the disease process itself.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80023-09 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Composition and Origins of the Extracellular Lining Layer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Research Chemist	LPFT	NIEHS
Others:	L. B. Gilmore	Biologist	LPFT	NIEHS
	G. George	Visiting Fellow	LPFT	NIEHS
	L. A. Dethloff	Bio. Lab. Technician	LPFT	NIEHS
	J. W. Spalding	Research Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The alveoli and distal airways of the lung are lined with an acellular layer of material which is essential for the maintenance of normal pulmonary functions such as gas exchange. The composition and origins of the acellular lining are being investigated. Current attention has been directed towards: (1) the biosynthesis and secretion of pulmonary surfactant and (2) the nature of the cytoplasmic organelles known as lamellar bodies. The objectives of this investigation are as follows: (1) to further develop methodology for the isolation of lamellar bodies from the lungs of rabbits (2) characterize the lamellar bodies according to phospholipid, protein and enzymic components, (3) to elucidate processes involved in the formation of the extracellular lining from secreted lamellar bodies and (4) to determine the distribution of pulmonary surfactant between intra- and extracellular pools.

PROJECT DESCRIPTION

METHODS EMPLOYED: Acellular lining material is obtained by lavaging the lung of rabbits via the trachea. Lamellar bodies from the cytoplasm of Type II cells are isolated on discontinuous sucrose gradients using differential centrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE Lamellar bodies are isolated from homogenized lungs of rabbits using a method developed in this laboratory involving the use of discontinuous sucrose gradients. The lamellar bodies were demonstrated to be morphologically intact and highly purified. The isolated structures were free of plasma membranes and mitochondria but contained about 17% protein attributable to endoplasmic reticulum. The yield of lamellar bodies was approximately 2%. The lamellar bodies were rich in lysosomal hydrolases. Future studies will attempt to determine compartmentalization of hydrolases within the lamellar body structure.

The fluidity of the major phospholipid in pulmonary surfactant, dipalmitoylphosphatecylcholine (DPPC) is inadequate to account for the spreading characteristics of surfactant at an air-water interface. Using electron paramagnetic resonance (EPR) and the probe 5-doxylmethylstearate we have studied the fluidity-modifying influence of the minor phospholipids in pulmonary surfactant on DPPC. Major fluidity changes in DPPC were obtained not with any of the minor phospholipids but with phosphatidylcholines containing unsaturated fatty acids in the 2-position indicating a potential role for the unsaturated components of surfactant. These studies will continue and include the influence of surfactant phospholipids on the rate at which DPPC may spread at air-water interfaces.

Methods have been devised for the estimation of the intracellular and extracellular pools of pulmonary surfactant. Extracellular surfactant is obtained by pulmonary lavage and quantitated according to phospholipid content. Intracellular surfactant is released from the tissue using decompression to disrupt the tissue, and isolated using discontinuous sucrose gradients. The intracellular pool of surfactant phospholipd in the lungs of rabbits is 1.66 ± 0.48 mg/g lung and the extracellular pool is 2.77 ± 0.49 mg/g lung. The kinetic relationship between these pools will be studied using radiolabelled phospholipid precursors.

SINGIFICANCE TO BIOEMDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The extracellular lining of the lung is vital for the maintenance of normal lung functions such as gas exchange. Inhaled toxicants such as the oxidant gases (e.g., ozone), particulate materials (e.g., silica) and chemicals (e.g., paraquat) appear to effect the acellular lining both qualitatively and quantitatively. The involvement of the acellular lining in the progression and mediation of some pulmonary diseases such as alveolar proteinosis appears certain. Unfortunately, the mechanisms which underlie these pulmonary diseases and agent-induced lung damage are not known. Elucidation of the biochemical processes which contribute to the formation of pulmonary surfactant and the acellular lining are a necessary step in the understanding of the disease process.

PUBLICATIONS

Bell, D.Y., Haseman, J.A., Spock, A., McLennen, and Hook, G.E.R.: Plasma proteins of the bronchoalveolar surface of the lungs of smokers and nonsmokers. Am. Rev. Resp. Dis. 124: 72-79, 1981.

Post. C.T., Squibb, K.S., Fowler, B.A., Gardner, D.E., Illing, J. and Hook, G.E.R.: Production of low-molecular weight cadmium-binding proteins in rabbit lung following exposure to cadmium chloride. Biochem. Pharmacol., in press, 1982.

Hook, G.E.R. and Gilmore, L.B.: Hydrolases of pulmonary lysosomes and lamellar bodies. J. Biol. Chem., in press, 1982.

Spalding, J.W., Ortner, M.J., Tombropoulos, E.G., Gilmore, L.B. and Hook, G.E.R.: Isolation and characterization of rabbit lung lamellar bodies. Exp. Lung Res., in press, 1982.

Hook, G.E.R.: The metabolic potential of the lungs. Clin. Pharmacol. Therap. 1: 117-146, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-06 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Neuroendocrine (Small Granule) Cells of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.P. DiAugustine	Research Chemist	LPFT	NIEHS
Others:	D. Vembu	Staff Fellow	LPFT	NIEHS
	I. Linnoila	Staff Fellow	Pathology	NCI

COOPERATING UNITS (if any)

Histology Laboratory, TRTP, NIEHS

LAB/BRANCH
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SECTION
Endocrinology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Solitary neuroendocrine-like (small granule) cells were examined for their distribution in the upper airways of the guinea pig and for their capacity to regenerate in xenografts maintained subcutaneously in the nude mouse. The solitary cells were present in the larynx at a frequency of 5.0 ± 1.8 (mean ± S.D.) per cent in guinea pigs of various ages: <1% of the glandular epithelial cells were of the neuroendocrine-type. In the trachea the frequency diminished caudally and constituted <1% in the bronchi. Only one population of solitary cells was observed throughout the study. The mucociliary epithelium in sections of the laryns (cricoid region) xenografted in nude mice was replaced by squamoid-like cells in one week. A normal-appearing mucociliary epithelium regenerated after one month. The full complement of small-granule cells was only attained in those regions exhibiting full maturation of the epithelium, indicating that such cells are derived from the airway epithelium as a late-stage event of cytodifferentiation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: General histological procedures were used for fixation and staining of tissue sections for light and electron microscopy. Epithelial cells were quantitated at the electron microscope at 11,000-fold magnification by counting only those cells that made direct contact with the basement membrane. Each cell was determined by the basal aspect of cytoplasm resting on the basement membrane and bordered laterally by membranes. Neuroendocrine-like cells were identified by their characteristic small granules.

For xenotransplantation, a transverse section (1-2 mm thick) of the cricoid region of the larynx was obtained from male guinea pigs and placed in medium 199 containing penicillin and streptomycin at ambient temperature. The section was xenografted subcutaneously in male Crl:nu/nu (BALB/c) BR mice for up to 56 days.

MAJOR FINDINGS AND PROPOSED COURSE:

Neuroendocrine-like cells of the guinea-pig upper airways. In the first phase of this study, the morphology and distribution of epithelial small-granule cells in the upper airways of the guinea pig were examined by electron microscopy. The pseudostratified epithelium of the cricoid region of the larynx contained basal cells, mucous cells, ciliated cells and cells with numerous small granules (130 ± 25 nm, mean diameter ± S.D.) of the neurosecretory type with a dense or granular core, a thin electron-lucent zone, and a trilaminar membrane. Other small granules within the same cell contained round electron-lucent zones of variable diameter within the core. The cells containing the small granules, provisionally categorized as neuroendocrine-like cells, appeared evenly distributed throughout the cricoid epithelium at a frequency of 5.0 ± 1.8 (mean ± S.D.) per cent in guinea pigs of various ages. Small-granule cells with identical morphological traits were also found in the tracheal epithelium but their frequency diminished caudally and constituted <1 percent of the epithelial cell population of the bronchi. Small-granule cells were also <1 per cent of the population in submucosal glands examined in the larynx. Only one type of small-granule cell was identified in guinea pigs ranging in age from 1-2 days to nearly 12 weeks.

In the second phase of this study, we examined the disposition of the small-granule cells after xenotransplantation of the guinea-pig cricoid into nude mice. By one week, the epithelium was replaced by flattened cells devoid of cilia, mucus-type granules and the characteristic small granules used to identify neuroendocrine-like cells. After one month, a mucociliary epithelium regenerated that contained small-granule cells in some regions at the frequency of the original donor tissue. The full complement of small-granule cells was only attained in those regions exhibiting full maturation of epithelial cells.

These findings suggest that the small-granule cells of the upper airways of the guinea pig are derived from a non-neuroendocrine population as a late-stage event of cytodifferentiation. This would agree with earlier studies in this laboratory which indicated by ^{3}H-thymdine-labeling of tracheal argyrophilic cells that neuroendocrine cells derived from some separate population of epithelial cells, possibly basal cells.

We have also tried to produce degranulation in vitro of cricoid neuroendocrine like cells with hyperopolarizing concentrations of K^+, and also with pilocarpine and the calcium ionaphore A23187. But, so far, no degranulation was apparent. This effort will conclude our electron miscroscopic examination of guinea-pig neuroendocrine cells.

The effects of the carcinogen diethylnitrosamine (DEN) on lung neuroendocrine-like cells and endocrine cells of other organs. Details of the experimental design are discussed in previous reports. We had earlier shown that DEN can readily cause hyperplasia of apparent neuroepithelial bodies in hamster lungs. We propose to determine whether this effect is highly selective for the lung (with the present dose regimen) or can extend to other (neuro)endocrine tissues, such as the intestine, pancreas, pituitary, adrenal and thyroid.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of the biolgoical properties of lung epithelial neuroendocrine cells and their humoral constituents should ultimately allow us to establish what function these cells have in the normal lung. Some lung-associated abnormalities may be initiated by improper levels of a regulatory peptide synthesized by a lung neuroendocrine cell. The experimental induction of lung neuroendocrine cell hyperplasia may indicate that certain lung tumors, namely bronchial carcinoid tumors and small cell carcinomas, may be preferentially formed by exposure to the nitrosamines in comparison to other classes of chemical carcinogens. Identification of lung-specific polypeptides should conceivably serve as a valuable plasma marker for early neoplastic responses of lung neuroendocrine cells.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80035-06 LPFT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Research Chemist	LPFT	NIEHS
Others:	J. Boyd	Technician	LPFT	NIEHS
	R. Mason	Research Chemist	LEB	NIEHS
	K. Sivarajah	IPA	LPFT	NIEHS
	D. Josephy	Guest Worker	LPFT	NIEHS
	J. Shirley	Technician	LPFT	NIEHS
	G. Reed	Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)

Drs. E. Zeiger and I. Robertson, Laboratory of Molecular Genetics; Dr. Fouts and Dr. Anderson, Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Caroline 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to study the oxidation of chemicals to toxic metabolites by prostaglandin synthetase (PGS) and to demonstrate the significance of this sytem in chemical-induced toxicity or carcinogenesis. BP-7, 8-diol metabolsim was studied in 10T1/2 cells where the PGs and NADPH-dependent oxidation can be compared. Stimulation of PGS resulted in elevation of metabolism and higher cell transformation. These studies suggest a potentially important role for PGS in BP-induced cell transformation. In addition, we studied the metabolism amines by PGS. Amines are metabolized to reactive intermediates by PGS.

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues, such as guinea pig lung and ram seminal vesicles, were used to examine the cooxygenation of xenobiotics during prostaglandin synthesis. The BP-7,8-diol metabolites were isolated from the incubation medium by extraction and separation by HPLC. Prostaglandins (PG) and thromboxane (TX) products were also isolated and quantitated. Tissue cultures were used to examine the interaction of electrophilic PB metabolites produced by PG synthetase with DNA and to determine if this interaction is related to cell transformation. HPLC was used to isolate and characterize the metabolites formed. Amine metabolites were measured by either spectrophotometry or by ESR techniques.

MAJOR FINDINGS AND PROPOSED COURSE: We have shown that the proximate carcinogenic metabolite of benzo(a)pyrene (BP), BP-7,8-dihydrodiol (BP-7,8-diol), is oxidized by PGS to BP-diol-epoxide I (BPDE-I), the ultimate carcinogenic metabolite of BP. Significant PGS dependent oxidation was observed in lung, skin, and kidney that have only 2% of the PGs activity of RSV microsomes. PGS and mixed function oxidase (MFO) dependent oxidation was compared in human lung. BP-7,8-diol was metabolized exclusively to BPDE-I by PGS and to both BPDE-I and II by MFO. The rates of oxidation were similar for both systems. PGS-dependent oxidation of BP-7,8-diol and the corresponding bay region diol of benzanthracene and chrysene produced mutagens as assayed in the Ames test (with Dr. Zeiger). These results suggest that cooxidation of polycyclic aromatic hydrocarbons (PAH) by PGS could play a role in the development of BAH-induced tumors in animals.

Metabolism of BP-7,8-diol by CH3 10T1/2 cells was also examined. These cells have both MFO and PGS activity. The addition of arachidonic acid (AA) to confluent dishes of 10T1/2 cells stimulated the metabolism of BP-7,8-diol to BPDE-I. The stimulation was dependent on concentration of the diol. Indomethacin inhibited the increase in metabolism on addition of AA. The addition of BP-7,8-diol to cells produced cell transformation. Increased transformation was observed on addition of AA, that was inhibited by indomethacin. Increased cell transformation was also dependent on the concentration of the diol. These results suggest that cooxidation by BP-7,8-diol can occur in intact cells even in the presence of MFO activity.

We have also examined a possible role of cooxidation in the development of BP-induced pulmonary tumors in AHe/J mice. This strain of mice has higher prostaglandin synthetase than other mouse strains examined and PGS dependent oxidation of BP-7,8-diol is greater than MFO dependent oxidation. A dose of aspirin was selected that inhibits PGS dependent but not MFO dependent oxidation. Tumors were also examined histologically (Dr. Boorman). The major pulmonary tumors appeared to arise from Type II cells with some derived from Clara cells. Aspirin treatment did not alter either the number of tumors, amount of DNA-adducts (Dr. Anderson, or types of tumors. Several explanations are possible for the lack of an aspirin effect. These are currently under consideration. We have recently compared MFO and PGS-dependent oxidation of BP-7,8-diol in isolated rat Clara and Type II cells (Dr. Fouts). MFO-dependent oxidation was greater than PGS-dependent oxidation by Clara cells while the reverse occured in Type II cells. Further studies are in progress with isolated tracheal cells in

order to assess the possible importance of BP-7,8-diol cooxidation. Studies with human bronchi are in the early stages. These studies will be done in collaboration with Dr. C. C. Harris, NCI.

In our initial studies, we examined a number of amines, including well-known substrates for MFO, for metabolism by PGS. Aromatic amines but not aliphatic amines were extensively N-demethylated to yield formaldehyde and N-demethylated substrate. A series of N-methyl substituted anilines were studied for N-demethylation by PGS. Very high rates of metabolism were observed except in aromatic amines containing nitro groups on the benzene ring. N-deethylation as well as N-demethylation was catalyzed by PGS. Metabolism was inhibited by indomethacin and dependent on arachidonic acid and peroxidatic in nature. O- and S-dealkylation of aromatic amines by PGS were not detected.

The mechanism of N-demethylation by PGS was studied using aminopyrine (AP) as a model substrate. During metabolism of AP a transient blue color was observed with PGS and was not observed with the MFO system, suggesting AP free radical formation (6). As aminopyrine cation free radical was detected by ESR. This radical was dependent on hydroperoxide or arachidonic acid and inhibited by indomethacin. Measurement of the radical and formaldehyde supported the hypothesis that aminopyrine is oxidized by PGS to a cation free radical which disproportionates to an iminium cation and aminopyrine. This cation is hydrolyzed to yield the methyl group as formaldehyde and demethylated amine. These results suggest a free radical mechanism for the N-demethylation of amines by PGS.

We have also studied the metabolism of acetaminophen (AE) by PGS. Acetaminophen produces hepatotoxicity in high doses but low dose chronic treatment produces kidney damage. Damage occurs in the papillary region of the kidney, which has high PGS activity and low MFO activity. AE was oxidized to a reactive intermediate that covalently bound to RSV protein. Binding was dependent on arachidonic acid or hydroperoxide, and inhibited by indomethacin and gluthionine in the incubation mixture. Purified PGS also oxidized AE to a reactive intermediate. Rabbit renal medullary microsomes also oxidized AE in the presence of arachidonic acid. No oxidation was observed in the presence of NADPH. We propose that in the renal medulla, AE is oxidized to a reactive intermediate, presumably the N-acetyl-p-benzoquinoneimine, that binds to protein and is associated with acetaminophen nephrotoxicity. Benzidine was shown to be oxidized by PGS to reactive intermediates that bind to DNA but little information is available concerning the identity of the metabolites. Metabolism of benzidine and several derivatives was studied using either horseradish peroxidase or PGS. The model chemical, 3,5,3',5'-tetramethylbenzidine (TMB) was oxidized to radical cation and charge-transfer complex composed of TMB and its two electron (di-imine) oxidation product. Benzidine and other benzidine derivatives appear to be oxidized by HRP and PGS by a similar series of reactions. The two electron oxidation products, the di-imines and a resonance structure of the nitrenium ions, are the proposed ultimate carcinogenic metabolites of this aromatic amine. We have also recently shown that the carcinogen 2-aminofluorene (2-AF) is metabolized to the corresponding hydroxyl amine by PGS. Metabolism of 2-AF was dependent on arachidonic acid or a hydroperoxide and inhibited by indomethacin. AF inhibited the peroxidatic oxidation of phenyl-butazone by PGS hydroperoxidase, suggesting that 2-AF is a reducing co-factor for PGS. The formation of N-hydroxy 2-AF was

indicated by isolation of the stable endproduct, axofluorene. The formation of mutagenic metabolites of amines was studied using bacterial test systems. 2-AF, benzidine, and β-naphthylamine produced mutagens while α-naphthylamine and 2-AAF were weakly positive. These results suggest that cooxidation could play a role in aromatic amine induced tumor formation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many xenobiotics are thought to exert their toxicity by means of reactive metabolites which are generated in vivo with very short half-lives. These metabolites react with tissue macromolecules to produce carcinogenesis, mutagenesis and teratogenesis. The prostaglandin synthetase system is found in most mammalian tissues and has particularly high levels of activity in the lung and kidney. Moreover, arachidonic acid can be released from its phospholipid storage sites by various types of stimulation, for example, irritation of lung tissue by inhaled pollutants. The subsequent metabolism of arachidonic acid by prostaglandin synthetase and the simultaneous co-oxygenation of xenobiotics could produce toxic metabolites.

PUBLICATIONS

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Lasker, J.M., Sivarajah, K., Mason, R.P., Kalyanaraman, B., Abou-Donia, M.D., and Eling, T.E.: A free radical mechanism of prostaglandin synthetase-dependent aminopyrine demethylation. J. Biol. Chem. 256: 7764-7767, 1981.

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Kalyanaraman, B., Mason, R., Tainer, B., and Eling, T.: The free radical formed during the hydroperoxide-mediated deactivation of ram seminal vesicles is hemoprotein-derived. J. Biol. Chem., 257: 4764-4768, 1982.

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UNIVERSITY OF CALIFORNIA - DAVIS, CALIFORNIA 95616
(222Y01-ES-0056)

TITLE: The Biology of Non-ciliated Bronchiolar Cells (Clara Cells) In Vitro

CONTRACTOR'S PROJECT DIRECTOR: Charles G. Plopper, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, Chief, LPFT

DATE CONTRACT INITIATED: July 1, 1980

CURRENT ANNUAL LEVEL: $35,000

PROJECT DESCRIPTION

OBJECTIVES: Non-ciliated Clara cells comprise a majority of the mucosal cell population in peripheral lung bronchioles. Morphologic and histochemical studies suggest Clara cells have a secretory function. The possible importance of Clara cells in toxic reactions of distal airways has come to light with the demonstration of high mixed-function oxidase (MFO) enzyme activity. Chemical toxins including carcinogens require MFO-catalyzed activation to form cytotoxic and/or carcinogenic metabolites. It has been shown that Clara cells respond adversely to hydrocarbons, ozone, nitrogen dioxide, hyperoxic conditions and cigarette smoke and are, therefore, likely to play a key role in the initiation of pulmonary injury and disease. Our objectives are to study the factors and mechanisms that induce and control bronchiolar Clara cell proliferation, differentiation and secretion. To facilitate these studies, we are developing in vitro cell and organ culture systems with which we can examine the biology of bronchiolar epithelium. Our research plan is, (1) to define the minimal cell and organ culture conditions necessary for maintenance of viable bronchiolar epithelium, (2) to define the specific culture conditions necessary for proliferation and determine the hormones required for differentiation and/or proliferation, and (3) to determine the influence of extracellular matrix components on airway cell growth and differentiation. Baseline steady-state parameters for bronchiolar epithelium will be determined from whole lung and isolated airways.

METHODS EMPLOYED: The first task in developing the in vitro models for Clara cell studies is to obtain quantitative information on the distribution of Clara cells and other cell types in conducting airways. The reactive frequency of the major cell types incldluing an assessment of the "non-ciliated" cell category in various sized airways of 10-20 week old, white New Zealand rabbits will be investigated. Cytochemical and ultrastructural characteristics of the non-ciliated cells will be established. The material to be investigated is a) isolated airways from adult rabbit lungs and b) intact adult rabbit lungs. Comparison between the two types of materials will allow judgement of the adequacy or inadequacy of the isolation procedures used to remove the lung parenchyma from the conducting airways. If needed, similar studies will be repeated in other species in order to select accessible, suitably sized airways with a majority of Clara cells for in vitro study. The cellular composition, viability and ultrastructural integrity of airways explants will be assessed qualitatively by light and transmission microscopy at different times of in vitro cultures. The cellular response to culture conditions in terms of proliferation and differentiation by hormones. (2) the mode and mechanisms of Clara cell secretion following

specific stimulation with hormones and drugs and, (3) the response of Clara cells to reactive gases.

MAJOR FINDINGS AND PROPOSED COURSE: In year one we demonstrated that one cell type (the Clara cell) is the nonciliated secretory cell lining all intrapulmonary and extrapulmonary airways in the rabbit lung. It's percentage of the epithelium varies from 17% in the trachea to 51% in the terminal bronchiole. The manuscript describing these findings has been submitted. For the current year, we have been addressing two questions: a) Is the trachea of other species lined by nonciliated cells resembling the Clara cells of that species? and b) Do the Clara cells of the rabbit airways secrete the same material in all airway generations? We have thus far compared the nonciliated cell ultrastructure of the tracheas of rats, hamsters, sheep and bonnet monkey, in addition to the rabbit. Mucous cells, with abundant nonhomogenous granules, basal nuclei and granular e.r., were 0.5% in rat, 0% in hamster, 4% in sheep, 13% in bonnet monkey and 1.2% in rabbit. Serous cells, with discrete granules and extensive GER, were 39% in rat. Clara cells with discrete electron-dense granules and extensive AER were found in hamster (41%), as well as rabbit (17%). Sheep had cells with extensive AER and discrete lucent granules (33%). In Bonnet monkey trachea, 11% of the epithelial cells had small electron-lucent granules, numerous polyribosomes, perinuclear golgi and moderate GER. The cytochemistry of rabbit tracheal cells at light microscope level shows that the goblet cells have sulfated mucopolysaccharides in their granules and the Clara cell granules do not react with any mucopolysaccharide stain. At e.m. level, Clara cell granules are of two types. Those with a light central core stain positively with dialyzed iron around the periphery, but not high iron diamine. They are probably protein in the core and sialylated glyconjugates around the rim. As a continuation of this project, we would like to define the functions of rabbit Clara cells at all airway levels and to compare functions at different levels. Three lines of investigation will be followed: 1) Complete characterization of secretory product using histo- and cytochemistry, 2) evaluation of roles of cell in airway repopulation following injury, and 3) identification of cytochrome P-450 system within cells of entire airway tree.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The structural complexities of whole lung make it difficult to investigate non-respiratory functions of various pulmonary cells, particularly those which occur in relatively low numbers in the majority of the small airways (e.g., Clara cells). Our specific aim is to develop *in vitro* model systems starting with whole isolated airways. The importance of studies of this nature lies in the fact that Clara cells are a known target for several environmental factors which may be the cause of pulmonary injury and disease. Yet, our understanding of the function and life cycle of these cells is still very rudimentary.

UNITED STATES DEPARTMENT OF ENERGY - OAK RIDGE OPERATIONS
Oak Ridge, Tennessee 37830
(222Y01-ES-80032)

TITLE: Susceptibility of Preneoplastic Epithelial Lesions to Toxic and Carcinogenic Insults

CONTRACTOR'S PROJECT DIRECTOR: A. J. P. Klein-Szanto, M.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

PERIOD COVERED: October 1, 1981 - June 1, 1982

CURRENT ANNUAL LEVEL: $165,000

PROJECT DESCRIPTION

OBJECTIVES: Our research was directed toward:

1) Establishing a two stage carcinogenesis model with DMBA as initiator and TPA as promoter using tracheal transplants.

2) Investigating the behavior of cell culture derived epithelia repopulating denuded rat tracheal transplants, and the effect of reexposing them to carcinogens or promoters.

METHODS EMPLOYED: The two stage carcinogenesis experiments were carried out by exposing rat tracheal transplants (t.t.) to a low initiating dose of 34 μg dimethylbenz(a)anthracene (DMBA) delivered during a two week period from beeswax cholesterol pellets (ratio 1:9) containing 100 μg DMBA. Immediately after this 14 day period of DMBA exposure, a beeswax-cholesterol pellet (ratio 8:2) containing 100 μg 12-O-tetradecanoylphorbol-13-acetate (TPA) was inserted in the tracheal lumina and left in for the duration of the experiment. Control groups (30-40 t.t. each) exposed to DMBA alone, TPA alone, and blank pellets, as well as 56 t.t. of the DMBA-TPA group were followed up for 24 months. Additional t.t. of these groups were analyzed at 2 weeks, 3, 10, and 12 months (see previous report).

The repopulation studies were carried out using the following cell lines: a) 3 cell lines originally treated with low doses of DMBA, b) 4 cell lines originally treated with low doses of MNNG, c) 3 cell lines originally treated with TPA, and d) 2 spontaneous "normal" respiratory epithelium cell lines.

Four weeks after inoculation of the cells into 60-80 denuded tracheas, they were exposed either to 0.05 μg MNNG in physiological saline, or to 100 μg TPA in a beeswax pellet. Control groups of approximately 20 t.t. were treated with a beeswax blank pellet or with saline. These treatments and procedures were carried out for each of the cell lines (total 960 tracheal transplants in 480 nude mice). One animal per cell line was sacrificed 4 weeks after inoculation of the cell lines. One hour prior to sacrifice, they were injected with ^{3}[H] thymidine. All groups were observed for tumor

development for up to 8 months after treatment or sacrificed when tumors reached a size of 20 mm in diameter (whichever came first).

MAJOR FINDINGS AND PROPOSED COURSE: The two stage carcinogenesis experiment has been finalized. DMBA alone induced a carcinoma incidence of 4.7%. The TPA alone treated animal group exhibited no tumors, and the DMBA-TPA exposed group showed a carcinoma incidence of 36%. It is noteworthy that this marked promotion effect becomes evident after the 14th month of promotion. Since our original project has been shortened due to budgetary restrictions, the studies on effects of dose rate of the initiator and on the effects of the interval between initiation and promotion on the type of lesions produced during two stage carcinogenesis were not carried out.

Most of the experiments in which cell culture derived epithelia which repopulate denuded tracheal transplant are still in progress. Two groups of tracheas repopulated with cell lines originally exposed to 165 μg DMBA (cell lines D55 and 4081) have been evaluated. In both cases the final tumor incidence 6-8 months after inoculation of the cells, between the MNNG reexposed tracheal transplants and the controls, were not significant. The latency periods were significantly shorter in the MNNG treated groups. Two of the normal cell line derived epithelia have been studied and although the controls also exhibited a few tumors, it is evident that both MNNG and TPA are able to accelerate the appearance of tumors, and especially MNNG, is able to induce the formation of a larger number of neoplasms.

The remaining groups will be sacrificed between June and September, 1983 and analyzed immediately.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The studies represent attempts to better understand the multifactoral nature of carcinogenesis, especially the influence of the combined or sequential effects of two or more chemical carcinogens and/or promoter in the respiratory tract. In addition, some new insight into novel morphological, cell kinetics and histochemical characteristics of preneoplastic lesions will help to better type, classify and eventually diagnose these putative cancer precursors. These types of experimental data are essential to design, in the future, rational approaches for diagnosis and intervention therapy in groups of individuals at high risk to develop lung cancer.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY
Summary Statement

Because the detection of environmental agents which affect reproduction or produce birth defects is unsure and the underlying biological mechanisms which account for these major health problems are unclear, the Laboratory of Reproductive and Developmental Toxicology seeks to bridge the gap between the most molecular aspects of reproductive biology and endocrinology and the more applied problems associated with the detection of hazardous chemicals, the extrapolation of laboratory data to man, and the estimation of human risk.

The Laboratory directs its research efforts to three major areas at the present time: (1) male and female reproductive processes; (2) teratogenesis; and (3) postnatal effects of gestational chemical exposure and is organized into three research program areas: Experimental Teratogenesis, Reproductive Toxicology, and Transplacental Toxicology. Extensive collaboration exists between these groups; thus, the current research projects for the Laboratory are listed below as integrated programs at various levels.

I. MOLECULAR LEVEL

A. Hormone-Related Gene Action in Accessory Sex Organs

- o Hormone regulation of prostate and seminal vesicle protein synthesis
- o Molecular characterization of androgen dependent rat seminal vesicle genes
- o Molecular cloning for androgen dependent rat prostate genes
- o Identification of a possible regulator DNA region in androgen responsive cell systems
- o Detection of v-oncogene expression in neoplastic human prostate cell lines and benign hyperplastic tissue

B. Biochemical Basis of Estrogen Action

- o Characterization of nuclear events involved in estrogen action in the mouse uterus
- o Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites
- o Analysis of proteins of the mouse uterus which are involved in the estrogenic response

C. Biochemical Basis for Craniofacial Teratogenesis

- o Glucocorticoid involvement in prenatal craniofacial development

- o Role of epidermal growth factor (EGF) in prenatal growth and differentiation

- o Biochemical basis of TCDD teratogenesis

II. ULTRASTRUCTURAL/CELLULAR STUDIES

A. Ultrastructural Changes as Predictors of Functional Abnormalities

- o Correlation of scanning and transmission electron microscopy observations with biochemical, histological, or functional changes in the male and female mouse genital tract

B. Toxication/Detoxication of Environmental Chemicals by Target Tissues Related to Reproduction

- o Polycyclic hydrocarbon metabolism by rodent testes

- o Increased TCDD induction of prostatic aryl hydrocarbon hydroxylase following treatment with DNA damaging agents

- o Development of a coupled system of microsomal enzymes and cultured rodent embryos to determine the role of metabolism in teratogenicity

- o Characterization of diethylstilbestrol metabolism and the elucidation of metabolic pathways which produce metabolites of differing biological activities

C. Toxicology of Early Development

- o Interspecies in vitro fertilization as an indicator of reproductive capacity

- o Chemical effects on preovulatory oocytes and preimplantation embryos

III. CELL/TISSUE/EMBRYO STUDIES

A. Sperm

- o Protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors

- o Interaction of plant lectins with sperm surface proteins during epididymal maturation

- o Monoclonal antibody analysis of sperm surface proteins during maturation and following chemical exposure

B. Cultured Embryos

- o Establishment and biochemical/physiological/morphological characterization of an *in vitro* system to grow and maintain whole rodent fetuses during critical periods of organogenesis
- o Studies of the Fetal Alcohol Syndrome

C. Isolated Development of Fetal Organs

- o Establishment and biochemical/physiological/morphological characterization of an *in vitro* system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity
- o Determination of oxidative metabolism of estrogenic chemicals in organ cultures of fetal tissues.
- o Morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice
- o Morphological characterization of long-term fetal tissue grafts
- o A model system to assess toxic effects on gametes, early development, pre- and post-implantation embryos, and fetuses

D. Cell Cultures as Model Systems

- o *In vitro* neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally related chemicals

IV. WHOLE ANIMAL STUDIES

A. Toxicology

- o The teratogenicity of anticonvulsant agents and structurally related chemicals
- o The teratogenicity of steroids and steroidal alkaloids
- o Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed *in utero* to diethylstilbestrol
- o Correlation of improved histopathologic assessment (semi-thin sections) of testicular damage induced by anticancer drugs with sperm counts and fertilizing capacity
- o Susceptibility of testicular tissue to early postnatal treatment with antineoplastic agents

B. Data Extrapolation to Man and Risk Estimation

- o Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans
- o Quantification of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk
- o Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons
- o Studies of human facial clefting

Summaries of these projects are presented below: details of the work appear in the individual annual report.

I. MOLECULAR STUDIES

A. Hormone-Related Gene Action in Accessory Sex Organs

Hormone regulation of prostate and seminal vesicle protein synthesis: Protein synthesis patterns analyzed by two dimensional (2D) gel electrophoresis in the prostate and seminal vesicle of castrated and testosterone stimulated rats indicate that a major group of secretory proteins in both organs is under androgen control. Both organs have a high concentration of poly(A^+)-mRNA which code in a wheat germ translation system for major polypeptides. Two major poly(A^+)-RNA's from prostate (labeled β and δ) code for the subunits of the major secretory product referred to as prostate binding protein or prostatein. These two prostate poly(A^+)-mRNA make up 30-40% of the total poly(A^+) of the prostate. A third major prostate poly(A^+)-mRNA (α) codes for a larger (22,000 dalton) secretory protein. Likewise, rat seminal vesicles have two major poly(A^+)-mRNA's (40%) which code for two major seminal vesicle proteins which are androgen responsive (IV and V).

Molecular characterization of androgen dependent rat seminal vesicle genes: Double-stranded complementary DNA for two major seminal vesicle poly(A^+)-mRNA's was prepared (ds cDNA to mRNAsv). The two seminal vesicle synthetic genes were enriched, A 11S poly(A^+)-mRNA comprised 40% of the total poly(A^+)-mRNA in the seminal vesicle. This 11S poly(A^+)-mRNA appears as two major and one minor band in agarose gel electrophoresis under denaturing conditions. The size of the two major poly(A^+)-mRNA bands are 650 NT (mRNAsv IV) and 580 NT (mRNAsv V). Poly(A^+)-mRNAs enriched for mRNAsv IV code in a wheat germ translation system for a polypeptide of 18,000 daltons, similar in size but slightly larger than the seminal vesicle secreted protein IV. Likewise, the mRNAsv V appears to code for a polypeptide of 14,000 daltons, again similar and slightly larger than the secreted protein V. Recent amino acid sequencing data indicates SVS protein IV is in fact 90 amino acids long, with a molecular weight of 10,000 daltons. The apparent higher molecular weight obtained from PAGE is due to the basic nature of these proteins (PI ≅ 9). The natural or chromosomal genes and flanking regions for SVS IV and SVS V have been characterized. The transcriptional unit for SVS IV is 1900 bp and for SVS V is about 1500 bp.

The 5'-flanking regions are presently being compared by sequence analysis and other method to help identify potential regulatory regions.

Molecular cloning of androgen dependent rat prostate genes: Double-stranded complementary DNA (ds cDNA 10-13s) to androgen dependent α, β, and δ poly(A^+)-RNA from ventral prostate was prepared using reverse transcriptase. The ds cDNA was treated with S_1 nuclease, tailed with terminal transferase and cloned in PBR322. Clones were identified by hybrid arrest translation of prostate mRNA which had been hybridized with insert DNA derived from candidate clones. Thus far three clones have been identified which carry the structural gene information for α, β, and δ polypeptides. Identification of each of these clones has been verified by hybridization of probes nick-translated from the respective inserts to ventral, lateral and dorsal prostate poly(A^+)-mRNA northern transferred to nitrocellulose filters. In the case of lateral and dorsal mRNA, no corresponding bands were found implying that these lobes of the rat prostate are biochemically different from the ventral prostate. Detailed restriction maps for each insert have been developed. Two signals from one million clones of the λ charon 4A rat gene library have been isolated. These phage clones contain an insert which codes for the 20,000 dalton translation product, α. The CfoI fragment containing the α gene is 5.2 Kb long and has 3 Ava II sites, cutting the insert into 4 fragments.

Human prostatic secretion: Human prostatic secretions obtained by rectal massage have been analyzed by two-dimensional electrophoresis. A prominent protein having a molecular weight of 17,000 daltons and an isoelectric point of 6.0 appears to be the major low molecular weight protein synthesized by the secretory cells of the human prostate. This protein may be homologous to one of the subunits of "prostatic binding protein" which is the major secretory protein of the rat ventral prostate and a marker for androgen-regulated gene expression in the normal human prostate.

B. Biochemical Basis of Estrogen Action

Characterization of nuclear events involved in estrogen action in the mouse uterus: The second translocation of hormone receptor complex to the nucleus after exposure to estrogen, which occurs in the mouse uterus, suggests two events in estrogen receptor action. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear event. The role of this event in estrogen action in the mouse reproductive tract, with particular regard to the actions of hormonally active environmental chemicals, is being studied. Receptor synthesis, RNA polymerase activities, DNA polymerase activities and glucose oxidation/utilization are also being investigated. Progesterone inhibition of uterine growth is also being studied to determine the biochemical events involved in estrogen growth promotion. Recent studies using steroid autoradiography raise the possibility that the second nuclear accumulation of estradiol-receptor complex may be related to its redistribution to different cell types within the tissue. Thus, estrogen action in the mouse uterus may involve sequential stimulation of various tissue compartments for its expression.

Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites: In order to determine whether the metabolism of DES results in biologically active or inactive metabolites, certain DES

metabolites and analogs were tested for estrogenic activity using both an in vivo bioassay and an in vitro receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity, while certain metaboites, e.g. β- dienestrol or ω-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation. The exception to these results were indanyl-DES and pseudo DES which are DES analogs as well as possible metabolites showing receptor binding comparable to DES, but with 20-100 times less biological activity. These studies have suggested a mechanism of differential genomic stimulation and altered clearance of analog receptor complexes from the target cell nucleus.

Analysis of proteins of the mouse uterus which are involved in the estrogenic response: Intracellular uterine proteins were labeled with [^{35}S] methionine using an in vivo stimulation/in vitro labeling technique. Two-dimensional gel electrophoresis was employed to detect any qualitative protein changes due to estrogen administration. Uterine tissue was subfractionated in order to determine what protein changes may have occurred in the individual cell fractions. Estrogen treatment resulted in the appearance of two uterine proteins in the 0.4 M KCl nuclear extract and a significant decrease in several proteins in the cytoplasmic fraction. Other protein labeling experiments using non-enzymatic separation of the individual uterine tissue compartments have indicated that some estrogen responsive proteins are unique to certain cell types. Studies are in progress to identify and determine a functional role for these proteins as well as to identify uterine secretory proteins as markers of uterine cell function and for their possible physiologic roles.

C. Biochemical Basis for Craniofacial Teratogenesis

Glucocorticoid involvement in prenatal craniofacial development: Depending on strain, glucocorticoids are potent inducers of cleft palate in experimental animals. The biochemical basis for this strain susceptibility appears to be due, in large part, to elevated levels of glucocorticoid receptors in the craniofacial tissues of fetal mice. Mesenchymal cells have been established in culture from the responsive A/J and nonresponsive C57 mouse palatal shelves. These cells are being examined in cell culture using a variety of parameters, including cell surface glycoproteins, to further define the biochemical basis for glucocorticoid induced teratogenicity.

Role of epidermal growth factor (EGF) in prenatal growth and differentiation: EGF is a potent polypeptide capable of stimulating proliferation and differentiation in a number of cell types in vitro and in vivo. Our recent studies have provided evidence for a fetal form of EGF that appears at midgestation and is presumably important for the development of a number of tissues including the secondary palate. Studies are in progress to characterize more extensively the biochemical properties of fetal EGF and compare it to the properties of adult EGF. EGF and glucocorticoids are known to act synergistically and therefore studies are in progress with both EGF and glucocorticoids in palate cell culture.

Biochemical basis for TCDD teratogenesis: Although, TCDD is the most potent teratogen known for experimental animals, it induces relatively few types of congenital anomalies including cleft palate. Strain differences in response to TCDD-induced cleft palate correlate well with elevated levels of TCDD cytoplasmic receptors in craniofacial tissues from sensitive fetal mice. Mesenchymal cell lines from palatal shelves have been established from the sensitive C57 and nonsensitive AKR strains to further explore the nature of TCDD-induced biochemical effects which could account for its teratogenesis.

II. ULTRASTRUCTURAL/CELLULAR STUDIES

A. Ultrastructural Changes as Predictors of Functional Abnormalities

Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histological or functional changes in the male and female mouse genital tract: Studies have demonstrated that scanning electron microscopy (SEM) provides a tool for the detection of early neoplastic changes. The surface ultrastructural features of the lumen of the female mouse genital tract was evaluated during the estrous cycle and during development in normal CD-1 mice. The hormone dependence of fine structural features of the cell surface was demonstrated in ovariectomized, hormone-treated females where various characteristics of intact animals were experimentally induced. Subsequent studies on DES-treated mice indicate that cell surface features are directly related to abnormal cell differentiation. Changes in the cell surface are correlated with alterations in the histological features of DES-exposed offspring. Transmission electron microscopic studies have shown that the most striking and reproducible ultrastructural lesion in the uteri of prenatally-DES treated females is an abnormal stromal-epithelial interface.

B. Toxication/Detoxication of Environmental Chemicals by Target Tissues Related to Reproduction

Polycyclic hydrocarbon metabolism by rodent testes: Polycyclic hydrocarbon activating and deactivating enzyme systems have been studied in the rodent testes. Both cell-free *in vitro* systems and the isolated perfused testis have been used. The two systems are being contrasted with regard to their ability to predict the ability of the testes of whole animals to metabolize exogenous chemicals and respond to enzyme-inducing agents.

Increased TCDD induction of prostatic aryl hydrocarbon hydroxylase (AHH) following treatment with DNA damaging agents: Oral TCDD pretreatment results in a 200 fold increase in prostatic AHH activity. This induction is potentiated 5 fold by prior intraperitoneal treatment with a DNA damaging agent such as procarbazine. DNA probes (cDNA) were used to monitor the effects of inducer and inducer plus DNA damaging agents on P-450 levels by measuring its mRNA synthesis. DNA damaging agents appear to make more TCDD binding sites at the Ah locus available to the inducing agent.

Development of a coupled system of microsomal enzymes and cultured rodent embryos to determine the role of metabolism in teratogenicity: Many toxic chemicals and most mutagens and carcinogens require metabolic activation of the substrate to an active form. Microsomal biotransformation enzymes have recently been coupled to embryos grown in culture in a manner analogous to the Ames test where an S-9 activating system is in contact with cultured Salmonella bacterial strains. It has been demonstrated that cyclophosphamide, an anticancer agent which requires metabolic activation, has no adverse developmental effects on cultured embryos unless the microsomal enzymes are present. Enzyme and cofactor requirements are being optimized for embryonic growth and enzymatic activity. Thus, a mammalian test system has been developed which might quickly predict environmental chemicals which are either direct or indirect acting teratogens.

Characterization of diethylstilbestrol metabolism and the elucidation of metabolic pathways which produce metabolites of differing biological activities: It has been demonstrated that peroxidase, an enzyme inducible in estrogen target tissues, is able to metabolize DES to its major metabolite, β-dienestrol. Bioactivation of DES was determined by the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from a target tissue, the mouse uterus. This peroxidase activating system was also studied in the hamster kidney, a non-genital target tissue for DES. The peroxidatic activity of prostaglandin synthetase was found to catalyze the oxidative metabolism of DES in vitro and in cell culture. The estrogenic activities of a series of DES metabolites and analogs were determined. Results of studies suggest that DES metabolism follows alternative pathways resulting in either metabolites which retain estrogenicity, lack activity or are of ambiguous activity. The latter class includes the indenestrol isomers and pseudo DES. These compounds have a comparable binding affinity to DES but are some 20-150 times less biologically active. Studies on the levels at which they fail to elicit a biological response include estrogen receptor translocation/clearance, DNA synthesis and mitosis. Determination of the biological significance of potentially activated metabolites of DES should aid in generalizations to other classes of estrogenic environmental chemicals.

C. Toxicology of Early Development

Interspecies in vitro fertilization as an indicator of reproductive capacity: Heterologous (human sperm/hamster egg) in vitro fertilization was used to assess fertilizing capacity of human males. These studies identified certain individuals as subfertile who have routinely determined normal sperm number, motility, and morphology. This approach demonstrated that the usual parameters for semen analysis are unsure predictors of male subfertility; only the most extreme semen abnormalities reliably predict human subfertility. Pregnancies occurring during the course of these studies have all been fathered by men with positive in vitro test results.

Chemical effects on preovulatory oocytes and preimplantation embryos: Following treatment of female mice with TCDD, the animals were mated to untreated males. Preimplantation embryos were then collected at different stages of development. Embryonic protein synthesis thesis was determined by culturing the embryos in a media containing ^{35}S-methionine. The newly synthesized proteins were extracted, solubilized, and separated using O'Farrell's two-dimensional gel electrophoresis method. The pattern of newly synthesized proteins at various embryonic stages were examined and compared to morphologic changes. Fragmented and collapsed embryos at the morula and blastocyst states were common. The mechanism of TCDD associated embryotoxicity and its effects on pre-ovulatory oocytes are being further studied.

III. CELL/TISSUE/EMBRYO STUDIES

A. Sperm

Protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify maturation factors: Two-dimensional protein patterns of epithelial cells from precaput and caput compared to cauda epididymis were strikingly different. Because these differences in the protein patterns were reproducible, they might serve as markers for detecting alterations in epididymal cell function with respect to sperm maturation and for determining sperm specific surface proteins of epididymal origin.

Interaction of plant lectins with sperm surface proteins during epididymal maturation. Lectins interact with specific glycoproteins on the cell surface membranes and, therefore, may be useful probes to monitor alterations in the number, distribution and mobility of cell surface receptors associated with sperm maturation. Thus, various lectins were employed to determine modifications in rat sperm surface proteins during testicular and epididymal maturation. Fluorescence-conjugated lectins were quantified visually. During passage from the testes, through the caput and cauda epididymides and vas deferens, binding to sperm of conconavalin A, wheat germ agglutinin, ricinus communis 120, and ulex europeus increased with increased sperm maturation. In contrast, soybean agglutinin sperm binding was greatest in testicular sperm and decreased with increasing maturity. Lectins appear to be useful probes to assess sperm maturation and perhaps identify sperm which have been affected by exogenous chemicals and rendered nonfunctional.

Monoclonal antibody analysis of sperm surface proteins during maturation and following chemical exposure: Monoclonal antibodies derived from hybrid cell lines provide highly specific probes that recognize unique determinants. Monoclonal antibodies to sperm surface proteins might be used to determine sperm membrane alterations associated with sperm maturation or chemically-

induced toxicity. Mice were immunized with rat sperm obtained from the precaput, caput and cauda epididymides. Splenocytes were obtained from minced spleen tissue and fused with myeloma cells. After cell fusion, the hybridoma supernatant was screened for relevant antibodies using an enzyme linked immunoabsorbent assay or FITC-conjugated rabbit anti-mouse IgG and IgM. Positive wells were further cloned. These monoclonal antibodies bind specific regions of the sperm surface and can be quantified by immunofluorescence. Following these developmental studies, the potential for monoclonal antibodies to identify chemically-induced sperm surface changes which might correlate with infertility will be assessed.

B. Cultured Embryos

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain whole rodent fetuses during critical periods of organogenesis: To aid in the laboratory assessment of teratogens and in the understanding of the molecular mechanisms underlying teratogenesis, an in vitro culture system for rodent embryos has been established. Rat conceptuses of pregnancy day 10 can be grown continuously for 96 hrs with extensive differentiation of major organs. Utilizing this embryo culture, nutritional and hormonal requirements for embryonic development are being studied, and the system's predictiveness for chemical teratogens is being tested. Extensive development of major organs occurs which is comparable to in vivo differentiation during the same period; organogenesis is highly sensitive to direct acting alkylating agents such as tetraethylene melamine (TEM). A microsomal enzyme activation system has been coupled to the embryo culture system which allows the detection of indirect acting teratogens such as cyclophosphamide.

Studies of the Fetal Alcohol Syndrome: The Fetal Alcohol Syndrome is a serious and frequent human birth defect. Our studies using whole embryo culture suggest that ethanol has a direct effect on the developing fetus. Investigations are in progress to more precisely define whether ethanol inhibits cellular proliferation and/or differentiation and to what extent its effects are restricted to the craniofacial region. Future studies will utilize scanning and transmission electron microscopy to more fully characterize the morphological effects of ethanol. In addition, immunofluorescent localization of various macromolecules, such as fibronectin, will be performed to assess the effect of ethanol.

C. Isolated Development of Fetal Organs

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity: The morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice has been established. Explants of fetal mouse gonads and genital tracts maintained in organ culture have been used to determine protein maps of developing tissues derived from DES exposed and unexposed animals. The biochemical studies are correlated with hormone response studies and morphological observations. Recent

studies have focused on growth factor requirements for proliferative response to estrogens in vitro. The oxidative metabolism of DES in the fetal genital tract organ culture system was determined; differences in metabolic patterns could be demonstrated in different fetal target tissues.

Morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice: A heterologous organ culture system including DES exposed or unexposed testes or Müllerian ducts has been used to determine the mechanism of Müllerian duct persistence and hyperplasia in DES treated males. Results suggest that the primary site of action is on the duct system rather than through a failure of the testis to synthesize or release Mullerian inhibiting Hormone.

Morphological characterization of long-term fetal tissue grafts: To overcome difficulties in long-term (greater than two months) maintenance of fetal tissues in vitro, cultured fetal gonads or genital tracts are carried as grafts in appropriately manipulated hosts. Under these conditions, fetal ovaries develop into functional gonads when grafted under kidney capsules. Some of the epithelial abnormalities observed in vivo can be seen in long-term explants of fetal vaginal tissues. Thus, the contribution of the postnatal environment to expression of prenatally induced abnormalities can be studied.

A model system to assess toxic effects on gametes, early development, pre- and post-implantation embryos, and fetuses: Male and female gametes can be exposed to environmental agents either in vitro or in vivo and then be used for in vitro fertilization. Conceptuses can also be recovered following mating of treated animals. The zygote is subsequently cultured and the early embryo (blastocyst stage) transferred to a pseudopregnant recipient. Using this approach, the following parameters can be monitored: sperm motility; in vitro fertilizing capacity; 4- and 8-cell stage formation; morula and blastocyst development; implantation success (resorbed/dead/live fetuses); pregnancy rate; and malformations. Thus, chemical effects on the sperm and ova, early development, preimplantation and postimplantation embryos, and birth defects can be studied.

D. Cell Cultures as Model Systems

In vitro neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally related chemicals: DES was shown to morphologically transform cells in culture at doses comparable to benzo(a)pyrene. The cells were tumorigenic when injected into appropriate hosts. Transformation was accomplished in the absence of measurable somatic mutation and of stimulated cell proliferation. Chemicals with structures capable of bioactivation by the peroxidase pathway were efficient transformants. Metabolism of DES to β dienestrol via this peroxidase pathway was shown to occur in this cell culture system.

IV. WHOLE ANIMAL STUDIES

A. Toxicology

The teratogenicity of anticonvulsant agents and structurally related chemicals: The teratogenicity of structurally related (cyclic imides) antiepileptic drugs was studied. Trimethadione, dimethadione, and paramethadione are derivatives of oxazolidinedione; diphenylhydantoin, ethootoin, and mesantoin are hydantoins; and phensuximide, methsuximide, and ethosuximide belong to the succinimide class of compounds. All the drugs and each of the basic chemicals produced embryotoxic and teratogenic effects. The malformation profile observed in the CD-1 mouse was similar to those described in humans following in utero exposure during anticonvulsant therapy. Comparative computer analysis of the dose-related increase in the incidence of malformations and adult lethality for each of the compounds indicated that the relative teratogenicity of the oxazolidinedione class of anticonvulsants was significantly greater than that of hydantoins and succinimides. A common mechanism of action associated with the imide structure is suggested by the fact that each of these drugs shares a common moiety and all are embryotoxic and teratogenic.

The teratogenicity of steroids and steroidal alkaloids: Glucocorticoids are potent inducers of isolated cleft palate in various mouse strains. The mechanisms appear to be receptor dependent and involve a preferential inhibition of craniofacial growth, which results in delayed palatal growth and failure of palatal fusion. Recent studies have shown that vitamin B_6 can substantially reduce the incidence of glucocorticoid-induced cleft palate. It appears that vitamin B_6 inhibits glucocorticoid binding to cytoplasmic receptors. The steroidal plant alkaloid, jervine, is known to be teratogenic in animals that feed on certain plants, such as veratrum californicum. Jervine is also teratogenic in certain strains of mice. Using radiolabeled jervine, we plan to explore its distribution and basic biochemical effects which account for its teratogenicity. In addition, labeled jervine will be used to investigate the possibility that its teratogenic action is dependent upon binding to the estrogen receptor.

Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed in utero to diethylstilbestrol: Prenatal exposure to DES in mice results in a dose-related decrease in fertility and genital tract abnormalities in the offspring of both sexes; in females, tumors of the vagina, cervix, uterus, and ovary were found. The stage of differentiation of the genital tract at the time of DES treatment was critical to the later expression of reproductive tract lesions. Induction of a benign vaginal lesion, adenosis, occurred later in development than the induction of the malignant vaginal lesion, adenocarcinoma. Immune function in both sexes was altered although not uniformly for males and females. These studies should be pertinent to the development of an animal model for similar human exposures.

Correlation of improved histopathologic assessment (semi-thin sections) of testicular damage induced by anticancer agents with sperm counts and fertilizing capacity. A recently developed histological approach involving organ perfusion, plastic embedding, and semi-thin sectioning, has been used to assess testicular damage induced by various anticancer drugs selected for their mode of action. Morphological findings are being correlated with alterations in sperm counts and disturbances in male reproductive function determined by serial mating. Sprague-Dawley male rats, 10 weeks of age, were treated once i.p. with the test agent (procarbazine). To properly fix the tissues, the testes were perfused initially with physiologic salaine to wash out the blood, followed by 5% glutaraldehyde. The hardened tissues were cut into small blocks and postfixed for 90 minutes in 1% osmium tetroxide and 1.5% potassium ferrocyanide to enhance contrast. The flat embedded specimens were cut at 1 μ thickness. Sperm were quantified by counting sperm heads in the testis and epididymides. Fertility was assessed by serial mating. Cytotoxicity and malformed germ cells are relatively easy to recognize morphologically, especially with these newer histological techniques. Only drastically reduced sperm counts lead to a decrease in fertility. Increased early pregnancy loss and abnormal development of implanted conceptuses observed during in vivo studies suggested genetic toxicity. Thus, it appears that even in a case of potent chemicals, a battery of different approaches including morphology, sperm counts, and serial mating are necessary to evaluate the complete spectrum of toxic actions which affect male fertility.

Susceptibility of testicular tissues to early postnatal treatment with antineoplastic agents: Unique susceptibility to chemical toxicity is critical to defining hazards and analyzing risks. Testicular development, because it involved both pre- and post-natal periods and includes the differentiation of various tissues, offers a number of possible targets for chemicals capable of perturbing biological processes. Unique tissue and cellular susceptibility was observed when anticancer agents were administered to rats at selected postnatal periods (days 6, 16, 24, and 45). Sertoli and Leydig cells replicate postnatally only early in life, and the cell populations are stable thereafter. Spermatogenesis is initiated shortly after birth. Vincristine (V) (all 4 treatment days) and cyclophosphamide (C) (day 16, 24, 45) delay puberty; C (16, 45), cytosine arabinoside (CA) (16, 24, 45) and V (6) increase reabsorptions; V (45) reduces sperm counts; and that C (16) and V (16, 24, 45) cause sterility in some of the animals. Reduced epididymal weight is found with C (16, 24) and V (24, 45). Histologic evaluations suggest an association between damage to a particular developing cell type and an observed dysfunction. Thus, dose and treatment schedule should be able to target Sertoli, Leydig, or spermatogenic cells. The possibility exists that with carefully selected doses and treatment regimens, laboratory animals could be produced which are deficient in one of these cell types. Such animals would be valuable models, especially to further explore the physiological role of the Sertoli cells.

B. Data Extrapolation to Man and Risk Estimation

Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans: Many of the genital tract lesions observed in mice exposed prenatally to DES have been observed in comparably exposed humans. For example, in the male, epididymal cysts, prostatic inflammation, sperm abnormalities, and cryptorchidism have been observed in both species; in females, vaginal adenocarcinoma has been seen in the prenatally-exposed mouse and human. Good examples of the utility of such studies is the report of retained testes in male mice derived from DES-treated mothers two years before a similar observation was reported in man and the report of dose-related subfertility in female mice two years before comparable reports in woman.

Quantification of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk: When percent malformations are expressed as a function of percent of the LD-50 dose, a family of curves are produced which distinguishes quickly those chemicals which are teratogenic at doses well below maternal toxicity. Thus, a more reliable reference point than dose is established. The relationhship between percent malformations and observable toxicity, such as CNS depression, is also being defined. Analyzing data concerning the teratogenicity of three anticonvulsant agents (trimethadione, diphenylhydantoin, and ethosuximide), it is readily apparent that the greatest clinical risk is associated with the use of trimethadione; the least with ethosuximide.

Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons: In the male gonads, factors which modify toxicity include the pharmacokinetic parameters governing the absorption, distribution, activation, and detoxication of toxicants; covalent binding to macromolecules; and DNA damage as well as DNA repair of damaged germ cells. All of these factors are being studied in our laboratory at the present time. The male germ cells are protected by a biological barrier comparable to that which retards the penetration of chemicals to the brain; permeability constants for the two are nearly identical. Toxication and detoxication processes are present in both the seminiferous tubule and interstitial cellular compartments. The balance of toxication/detoxication processes apparently favors the germ cells; detoxication reactions are relatively more abundant in the seminiferous tubules. Unscheduled DNA repair has been demonstrated in spermatogonia and spermatocytes; spermatids and sperm lack DNA repair capability. The DNA repair capacity associated with spermiogenic cells appears to be dose-dependent and saturatable. Understanding the pharmacokinetic characteristics of the blood-testis barrier, toxication and detoxication mechanisms as well as DNA repair systems in male gonads will allow a better understanding of species comparison, of reproductive and genetic toxicity, and will increase the reliability of extrapolating laboratory animal data to man and estimating human risk.

Studies of human facial clefting: Cell lines have been established from the dermal fibroblasts from a number of normal and abnormal individuals with facial clefting ranging from 3 months to 37 years of age. Preliminary studies suggest alterations in the level of glucocorticoid receptors in these cells which presumably could indicate a defect which might have adversely affected fetal development. Studies are in progress to determine the level of receptors for other hormones and growth factors and to examine these affected cells for sensitivity to various known or suspected human teratogens. This information may eventually prove useful in genetic screening and counseling for individuals predisposed to genetic and/or environmentally-caused birth defects.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international levels. Laboratory scientists appreciate the expertise and resources concentrated in nearby universities. Moreover, we have not hesitated to establish collaborative efforts with scientists throughout the world to increase the quality intramural programs.

Dr. Carter is involved in a collaborative project with Dr. Martin Resnick, Department of Urology, Bowman-Gray School of Medicine; Dr. Don Mickey, Department of Urology, University of North Carolina; and Dr. Julius Horoszewicz, Roswell Park Memorial Institute. Dr. Resnick provides BPH tissue and primary human prostate carcinoma tissue; Dr. Mickey provides neoplastic prostate cell lines and primary tumor tissue; and Dr. Horoszewicz provides androgen-responsive human prostate cell-lines.

Dr. Lee is involved in a collaborative study using cDNA probes of the potentiation by procarbazine tetrachlorodibenzo-p-dioxin induced prostatic aryl hydrocarbon hydroxylase activity. Drs. M. Negishi and D. W. Nebert, NICHD, collaborated on the kinetic studies of mRNA synthesis in rat prostatic tissues. Dr. H. Eisen, NICHD, assisted with TCDD receptor affinity and quantitation in the prostate glands of Ah+ and Ah- strain of mice.

Dr. McLachlan continues to be involved in collaborative research with the Institute for Pharmacology and Toxicology, University of Wurzburg, Germany; Department of Obstetrics/Gynecology, Duke University Medical Center; and Department of Comparative Medicine, Bowman-Gray School of Medicine. These collaborative projects involve a detailed exploration of transplacental toxicity of DES and other hormonally-active chemicals.

Dr. McLachlan and Dr. Korach continue to participate in collaborative studies regarding X-ray crystallography of DES metabolites with Dr. William Duax, Medical Foundation of Buffalo.

Dr. Korach is involved in a collaborative project with Dr. Charles Eldridge, Bowman-Gray School of Medicine, regarding the IRAs of steroid hormones in the mice.

Dr. Korach is also participating in a collaborative project with Dr. Indu Parikh and Dr. Bruno Moncharmont, Burroughs-Wellcome Research Labs. They are studying estrogen receptor antibody immunoassay and antibody preparation for estrogen receptor.

Dr. Pratt continues his collaborative studies with Dr. Ken Brown, National Institute for Dental Research; Dr. G. Cunha, University of Colorado Medical School; Dr. T. Yoneda, University of Osaka, Japan; and Dr. L. Dencker, University of Uppsala, Sweden.

Dr. Kim is involved in collaborative studies with Dr. James Bawden of the Dental Research Institute of the University of North Carolina at Chapel Hill concerning localization of labeled teratogens in the rodent embryo.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer recognition and scientific relevance of current Laboratory programs is the frequency that LRDT scientists organize and participate in "state of the art" symposia and are asked to serve on various committees attempting to provide meaningful directions in environmental health research. Descriptions of representative examples for the Laboratory are given below:

Dr. Dixon has a large number of committee and program assignments which augment and are relevant to the NIEHS mission such as President of the Society of Toxicology, member of the Toxicology Advisory Board at Raven Press in New York City, member of the Advisory Committee for the Burroughs-Wellcome Toxicology Scholar Award, Councilor for the Section on Environmental Health Sciences of the Pan American Medical Association, representative to the International Union of Pharmacology Scientific Committee on Problems of Environment, and member of the Toxicology Review Panel of the World Health Organization's Expanded Programme of Research Development and Research Training in Human Reproduction. He has also co-organized two Workshops sponsored by the World Health Organization's International Program on Chemical Safety. These Workshops on "Methods for the Integrated Evaluation of Risks for Progeny Associated With Prenatal Exposure to Chemicals" were held in Leningrad, USSR, and Prague, Czechoslovakia. In addition, Dr. Dixon remains a co-organizer of the Target Organ Toxicity Symposia Series. Proceedings of these Symposia are published in Environmental Health Perspectives and include symposia on liver, kidney, lung, gonads, development, nervous sytem, cardiovascular system, intestines, blood, endocrine system, immune system, and eye, ear and other special senses. He is presently Editor-In-Chief of the Target Organ Toxicity Mongraph Series published by Raven Press.

Dr. McLachlan continues to be a member of the DHHS Task Force on DES toxicity and has been advisor to NIOSH and the FDA on the toxicity of estrogens.

Dr. Pratt has been involved in the organization of an International Symposium on In Vitro Screening Tests for Teratogenicity, and Environmental Protection Agency Meeting on Risk Assessment.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. LRDT scientists are frequently asked to review manuscripts for journals oriented toward Biochemistry, Pharmacology, Toxicology, and Teratology. Dr. Dixon is on the Editorial Boards of Environmental Health Perspectives, Toxicology and Applied Pharmacology, Journal of Pharmacology and Experimental Therapeutics, The Encyclopedia of Pharmacology and Therapeutics, Journal of Environmental Sciences and Health, Journal of Environmental Pathology and Toxicology, Journal of Toxicology and Environmental Health, and Funadamental and Applied Toxicology.

Dr. McLachlan is on the Editorial Board of the International Journal for Biological Research in Pregnancy.

Dr. Korach is on the Editorial Board of Environmental Health Perspectives and serves as a reviewer for Endocrinology, Science, Biochemica Biophysica ACTA, and Biology of Reproduction.

Dr. Pratt is on the Editorial Boards of Differentiation, Teratogenesis, Mutagenesis and Carcinogenesis, and Journal of Craniofacial Genetics and Developmental Biology.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Reproductive and Developmental Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training activities, including accepting adjunct appointments at nearby universities, supervising graduate student research, developing graduate courses in environmental health, and participating in the Fogarty International Center's Visiting Program.

Dr. Harris and Dr. McLachlan are members of the UNC Cancer Center.

Laboratory scientists have also been active in the training of graduate students. Graduate students are working in the laboratories of Drs. McLachlan and Pratt.

Drs. Dixon, McLachlan, Korach, and Pratt have lectured at UNC, Duke and/or Burroughs-Wellcome in areas of their research expertise.

Dr. Pratt has organized a graduate level course at the University of North Carolina entitled "Developmental Toxicology and Teratology." Dr. Dixon is an invited lecturer for the course.

Dr. Pratt is an Adjunct Professor at the University of North Carolina in the Department of Anatomy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70010-06 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Normal and Abnormal Embryonic Development*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Pratt	Research Chemist	LRDT	NIEHS
OTHER:	C. S. Kim	IPA	LRDT	NIEHS
	E. H. Goulding	Biological Lab Technician	LRDT	NIEHS

COOPERATING UNITS (if any)

NIDR, NIH

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) To culture postimplantation embryos in order to aid the laboratory assessment of potential teratogens; (2) to define the mechanism of teratogenesis at the morphological, biochemical and molecular level using in vivo studies as well as cultured embryos exposed to teratogens of interest; (3) to determine the role that various hormones and growth factors play in early postimplantation embryonic development.

(*Formerly, Study of Developmental Disorders Using Cultured Embryos)

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) In vitro development of rat conceptuses was unaffected by the addition of cyclophosphamide alone to the medium. However, addition of a combination of cyclophosphamide and rat liver cells produced deleterious effects on embryonic development indicating metabolic conversion of cyclophosphamide into active components which presumably induce abnormal development. Thalidomide along with rabbit liver cells also produced abnormal development, especially in the forelimb buds.

(2) Exposure to ethanol retards growth and differentiation in cultured rat embryos during organogenesis. The development of untreated embryos is indistinguishable from growth in utero. These data suggest that the hypoplastic features of children born to chronically alcoholic mothers are due, at least in part, to a direct action of ethanol, which causes reduced embryonic cellular proliferation early in gestation.

(3) Exposure to jervine, a steroidal alkaloid which produces cranial-facial anomalies in vivo in the rodent, elicits a dose- and stage-dependent malformation in cultured embryos. This provides evidence that jervine acts directly on the developing embryo in vivo to produce malformations. Jervine appears to elicit these effects by interfering with the development of specific cranial neuroepithelial cells.

(4) Exposure of cultured mouse embryos to epidermal growth factor, including microinjection into the yolk sac, stimulates embryonic growth suggesting that EGF is involved in early postimplantation development.

The proposed course of this project involves developing procedures for in vitro growth and differentiation of embryos under more defined conditions. Effects of direct or indirect acting teratogens on the in vitro embryo development will be studied, and conditions for metabolic activation of environmental agents by liver cells as well as the maternal involvement in the production of actual teratogens will be explored in-depth. This developmental toxicity model will be further developed and the effects of selected chemicals will be observed in vitro and correlated with in vivo effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Exposure to adverse environmental factors is presumably a major cause of developmental abnormalities in man. The laboratory prediction of teratogenic chemicals is unsure and mechanism of teratogenesis unclear. An in vitro system for culturing embryos would provide an opportunity to study cellular and molecular processes associated with normal and abnormal development. Such an in vitro model would also be useful in predicting the toxic potential of environmental agents and in understanding the biological mechanisms by which chemicals may disrupt early development and produce birth defects.

PUBLICATIONS

Sim, F, R. P., Matsumoto, N., Denny, D., Goulding, E., and Pratt, R. M.: Specific developmental defects induced by jervine in whole embryo culture. Teratology (In press).

Brown, N. A., Schull, G., Kao, J., Goulding, E. H., and Fabro, S.: Teratogenicity and lethality of hydantoin derivatives in the mouse. Tox. Appl. Pharm. (In press).

Schmid, B. P., Goulding, E. H., Kitchin, K., and Sanyal, M. K.: Assessment of the teratogenic potential of acrodein and hydrophosphamide in a rat embryo culture system. Toxicology (In press).

Brown, N. A. and Fabro, S.: Quantitation of rat embryonic development in vitro: A morphological scoring system. Teratoology 24: 65-78, 1981.

Kao, J., Brown, N. A., Schmid, B., Goulding, E. H., and Fabro, S.: Teratogenicity of valproic acid: In vivo and in vitro investigations. Terat. Carcin. Mut. 1(4): 367-382, 1981.

Fabro, S., Schull, G., and Brown, N. A.: Relative teratogenic index and teratogenic potency: Proposed components of developmental toxicity risk assessment. Terat. Carcin. Mut. 2: 61-76, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70011-02 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of Normal and Abnormal Craniofacial Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Pratt	Research Chemist	LRDT	NIEHS
OTHER:	R. I. Grove	Post Doctoral Fellow	LRDT	NIEHS
	W. D. Willis	Biologist	LRDT	NIEHS
	C. S. Kim	IPA	LRDT	NIEHS

COOPERATING UNITS (If any)
Dental School, University of Osaka, Osaka, Japan
Department of Toxicology, Uppsala University, Uppsala, Sweden
Dental School, University of British Columbia, Vancouver, Canada

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Growth and differentiation of craniofacial tissue is complex and involves multiple cell types interacting with each other and a number of hormones and growth factors. Of particular interest are steroids such as glucocorticoids, and epidermal growth factor (EGF). These agents are teratogenic at high doses whereas physiological levels of EGF and glucocorticoids are required for normal growth and differentiation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) Human embryonic palatal mesenchymal cells (HEPM) were found to be a good model system in which to determine the factors regulating certain aspects of craniofacial development. These cells respond to pharmacological levels of glucocorticoids such as dexamethasone (DEX) by a pronounced inhibition of growth. This growth inhibition presumably is the major effect of cortisone responsible for cleft palate. Our studies show that dexamethasone produces a number of interesting alterations in cell surface proteins and lipids and future studies will be aimed at correlating to the growth inhibitory effects of DEX. These HEPM cells can be grown in a completely defined medium and our studies show that growth is highly dependent upon the presence of EGF, which is thought to be involved in a fetal form in normal palatal development. These HEPM cells, which are established in culture appear to be an excellent model in which to further explore the biochemical and molecular basis for normal and abnormal craniofacial development.

(2) The growth of HEPM cells is also being tested as a possible short-term screening assay for potential teratogens. The growth inhibitory properties of many teratogens are easily determined using the HEPM cells. This assay along with other types may provide a battery of tests to be used for prescreening teratogenic agents in the environment.

(3) The mechanisms of TCDD-induced cleft palate is currently under investigation. In contrast to the glucocorticoid-induced cleft palate, TCDD does not inhibit palatal shelf growth or contact but appears to interfere with some aspects of epithelial differentiation. Studies are in progress to determine the precise localization of labeled TCDD in the developing embryo. Recent studies have shown that the epithelial cells of the palatal shelf can be cultured with good growth and differentiation of the various types of epithelial cells in the absence of palatal mesenchymal cells. Studies are in progress to determine the influence of a number of growth factors, hormones, and teratogens (TCDD) on palatal epithelial cell growth and development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Facial clefting is a significant human congenital malformation and is one of the most frequently observed abnormalities. The etiology of facial clefting is complex, but appears to involve both genetic and environmental factors. The mouse is an excellent model since various inbred strains are exclusively sensitive to craniofacial teratogens. Our studies involve both whole animal as well as cell and organ culture to further define the complex interactions between genetic and environmental factors. Information from our studies may lead to better genetic counseling and drug avoidance during pregnancy and may reduce the frequency of congenital facial clefting in the human.

PUBLICATIONS

Diewert, V. and Pratt, R. M.: Cortisone-induced cleft palate in the A/J mouse: Failure of palatal shelf contact. Teratology 24: 149-162, 1981.

Sim, F. R. P., Salomon, D. S., Nylen, M. V., and Pratt, R. M.: Tumor promotor (TPA) mimics EGF-induced precocious tooth eruption in the rodent. Terat. Mut. Carcin. 1(4): 361-366, 1981.

Yoneda, T. and Pratt, R. M.: Mesenchymal cells from the human embryonic palatal are highly responsive to EGF. Science 213: 563-565, 1981.

Yoneda, T. and Pratt, R. M.: Glucocorticoid receptors in palatal mesenchymal cells from the human fetus: Relevance to human cleft palate formation. J. Craniofacial Genetics and Developmental Biol. 1(4): 401-423, 1981.

Yoneda, T., Goldman, A. S., Van Dyke, D. C., Wilson, L. S., and Pratt, R. M.: Decreased number of glucocorticoid receptors in dermal fibroblasts from individuals with facial clefting: A preliminary report. J. Craniofacial Genetics and Developmental Biol. 1(2): 229-234, 1981.

Yoneda, T. and Pratt, R. M.: Interaction between glucocorticoids and EGF in vitro in the growth of palatal mesenchymal cells from the human embryo. Differentiation 19: 194-198, 1981.

Eto, K., Figueroa, A., Tamura, G., and Pratt, R. M.: Induction of cleft lip in cultured rat embryos by localized administration of tumicamycin. J. Exp. Embryol. Morphol. 64: 1-9, 1981.

Dencker, L. and Pratt, R. M.: Association of the presence of the Ah receptor in embryonic tissues from mice sensitive to TCDD-induced cleft palate. Terat. Carcin. Mut. 1: 399-406, 1981.

Pratt, R. M., Grove, R. I., and Willis, W.: Prescreening for environmental teratogens using cultured mesenchyme cells from the human embryonic palate. Terat. Carcin. Mut. (In press).

Pratt, R. M.: Mechanisms of chemically-induced cleft palate. Trends in Pharmacological Sciences (In press).

Yoneda, T. and Pratt, R. M.: Vitamin B_6 reduced cortisone-induced cleft palate in the mouse. Teratology (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-06 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular Mechanism of Androgen Mediated Gene Expression in Male Sex Accessory Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Harris	Senior Staff Fellow	LRDT	NIEHS
OTHER:	B. A. Dickson	Biologist	LRDT	NIEHS
	D. B. Tully	Graduate Student	LRDT	NIEHS
	C. S. Teng	Guest Worker	LRDT	NIEHS
	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to study the mechanisms involved in androgen mediated gene expression in the rat and mouse seminal vesicle. The genes for the major seminal vesicle secretion protein IV and V (SVS IV and V) are under detailed study. Bacterial clones have been identified and characterized which contain inserts for cDNAs SVS IV and SVS V from rats. The cDNA IV and V were shown to have unique sequences. Sequence comparisons show very little homology in the coding regions. A region, in the 3'-non-coding portion of the mRNA, is, however, almost 80% homologous. The androgen induction of IV and V mRNA in castrate rats was measured using the respective probes. The chromosomal genes for IV and V were isolated from a rat gene library and the SVS IV gene was shown to be about 1.9 kb with two introns. Another observation is that the SVS IV gene has an insertion of approximately 200 bp in the second intron. The insertion site is flanked by 20 bp direct repeats. A major repetitive element (100,000 copies/genome) was shown to be on the 3' end of the SVS IV gene. A palindromic structure at -117 bp from the CAP site was identified and shown to be S1-nuclease sensitive in the supercoiled state. DNase I and S1-nuclease sensitive sites in chromatin structure around the SVS IV gene are presently being defined.

PROJECT DESCRIPTION

METHODS EMPLOYED: E. coli. RR1 containing plasmids with SVS IV or V inserts were grown and amplified in L-broth containing chloramphenicol. Plasmids were isolated using CsCl-propidium di-iodide centrifugation. Restriction digestion and agarose or acrylamide electrophoresis were done by standard procedures. The wheat germ translation system was used in hybrid-arrest experiments. The dot blot hybridization assay was used with nitrocellulose, and RNA in various concentrations baked onto the filters. Probes of cloned DNA fragments were prepared with ^{32}P-NTP and nick-translation. Southern and Northern hybridizations were performed by standard techniques. Subcloning in pBR325 was performed by T4 ligation of EcoR1 fragments, chloramphenicol selection, and Grunstein-Hogness hybridization procedures. Phage clones were screened after amplification using the Benton-Davis procedure. Phage isolation and purification was performed by discontinuous CsCl banding centrifugation procedures. Phage DNA was isolated by standard procedures. DNA sequencing was performed by the Maxam and Gilbert procedure and the M13 cloning/dideoxynucleotide methods. Detection of supercoiled plasmid and subsequent linearization with S1-nuclease were analyzed using Tris-acetate buffer in agarose gel electrophoresis. Isolation of nuclei was performed using the standard heavy sucrose method. Isolation of other vectors was performed as above using the appropriate antibiotics.

MAJOR FINDINGS AND PROPOSED COURSE: Rat seminal vesicle genes whose expression is androgen dependent are being characterized. The structural genes (cDNAs) for the two major seminal vesicle secretory proteins IV and V (SVS IV and SVS V) have been cloned and identified by restriction mapping, hybrid arrest translation assays, and direct DNA sequencing. The complete DNA sequence for SVS IV coding region and SVS V coding region has been determined. The coding regions have almost completely diverged, except a region of about 10 amino acids at the C-terminal in which there is ~60% homology. Interestingly, a region of about 55 bp in the 3'-non-coding region has over 75% homology. This region may be involved in the relatively long half-life of these mRNA's, even after castration. The induction of mRNA IV and mRNA V were compared in 4 week castrate males given daily injections of testosterone. To summarize, the two mRNAs are coordinately induced up to 72 hours, but the level of mRNA V is in general about one-half that of mRNA IV. The genomic SVS IV has been almost completely sequenced. A 150 bp sequence of the 5'-flanking region is also known. A palindromic structure at ~117 bp from the transcription initiation site has been shown to exist in supercoiled DNA plasmid, containing the insert. This site has potential regulatory functions by several criteria. The genomic SVS V gene(s) and flanking regions have also been isolated and are presently being sequenced to compare to the SVS IV gene flanking region. The two genes seem to be coordinately expressed under the influence of androgens. A major rat repetitive element (100,000 copies/genome) was identified on the 3' flanking region of the SVS IV gene. This may define the 3' boundary of a larger DNase I sensitive domain containing several of the seminal vesicle secretory genes. S1-nuclease sensitive and DNase I hypersensitive sites in the chromatin structure have initially been mapped in nuclei isolated from castrate and testosterone treated animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Using the technology presently available, a relatively detailed model of how steroid hormones act on their target tissues can be obtained in the near future. With this information, we can perhaps better predict the effect of various environmental chemicals on the variety of steps involved in gene function.

PUBLICATIONS

Korach, K. S., Harris, S. E., Carter, D. B.: Protein patterns of the mouse uterus after estrogen exposure: Analysis by two-dimensional gel electrophoresis. Mol. Cell Endocrinol. 21: 243-254, 1981.

Carter, D. B., Newbold, R. R., Harris, S. E., and McLachlan, J. A.: Molecular differentiation of the mouse genital tract: Protein synthesis in fetal and immature female reproductive tract. Biol. Reprod. (In press).

Harris, S. E., Mansson, P. E., Tully, D. B., and Dickson, B. A.: The seminal vesicle secretion IV gene: Possible allelic difference due to a small insertion in an intron. Proc. Natl. Acad. Sci. USA (In press).

Carter, D. B., Yamada, K., Monahan, J. J., and Harris, S. E.: Investigation of androgen-dependent mRNA from rat ventral prostate using cloned cDNA. J. Biol. Chem. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70047-06 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Prostate as a Model System to Study Normal and Abnormal Gene Expression in the Rat and Human

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Carter	Senior Staff Fellow	LRDT NIEHS
OTHER:	K. Yamada	Visiting Fellow	LRDT NIEHS
	S. E. Harris	Senior Staff Fellow	LRDT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three clones for structural genes coding for proteins secreted from the rat ventral prostate have been identified by hybrid arrest translation. The three cloned inserts have been mapped with restriction endonucleases, and the homologous mRNAs have been shown to be induced by androgens. A rat gene library carried in λ phage charon 4A has been screened for the genomic sequences corresponding to two of the secreted proteins. Two signals have been purified from one million phage clones and the inserts subcloned into PBR325. A Cfo I fragment 5.2 kb has been characterized from both signals which codes for part of a 20,000 dalton secreted protein. Sequencing of this gene is underway.

Human and rat ventral secretory proteins were compared by two-dimensional gel electrophoresis of human and rat prostate secretions. A major low-molecular weight protein is synthesized by both rat and human prostates and has similar but different molecular weight/isoelectric point coordinates (13,000/5.6 rat, 17,000/6.0 human). Homology of these proteins at the cDNA level is under investigation.

PROJECT DESCRIPTION

METHODS EMPLOYED: Poly(A^+)-mRNA from rat ventral prostate was fractionated by SDS-sucrose gradients and double-stranded complementary DNA (cDNA) was synthesized using reverse transcriptase for both strands. After "tailing" the cDNA with deoxyadenosine residues, the mixture was annealed to dT tailed plasmid PBR322.

E. Coli RR1 were transformed with the recombinant plasmids, and ampicillin sensitive/tetracycline resistant clones were selected for plasmid purification. Hybrid arrest translation of total poly(A^+)-mRNA hybridized with restriction endonuclease digested plasmid DNA isolated from various rat clones was used to identify three plasmids containing the coding information for three rat prostate secretory proteins. The rat gene library was screened with a ^{32}P-nick translated α cDNA probe and signals obtained by the Benton-Davis method. Subclones of inserts from purified phage clones were established in PBR325. Mapping of the PBR325 insert with nick translated fragments of the cDNA probe is being carried out using southern procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Restriction maps of cloned structural genes for three of the major secretory proteins from rat ventral prostate have been developed. Purified inserts from these plasmids have been obtained from agarose gels. Nick-translated probes have been synthesized from the pure inserts and used to follow the androgen regulation of the homologous mRNA's in the rat ventral, lateral, and dorsal prostates. In addition, nick-translated probes to two of the inserts have been used in a primary screening of the rat gene library carried in λ phage charon 4A. The natural gene for one of these androgen dependent secretory products has been isolated by repeated rounds of screening. Sequencing of the insert is underway and comparison with the sequence of the cDNA clone should elucidate the structure of the gene.

Various human prostate cell lines are being investigated for androgen-regulated expression of the major (17,000 dalton) secreted protein. In addition, investigation of expression of the v-oncogenes abl, bas, myc, and ssv in DU-145, PC-3, and LnCap cell lines is underway. The expression of the ssv v-oncogene has been detected in the PC-3 cell line, a bone metastasis of prostatic adenocarcinoma.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The rat ventral prostate is dependent on androgen for normal secretion and function. This organ has a set of genes which produce large quantities of particular messenger RNAs and their corresponding structural proteins, thus making this system ideal for studying the molecular mechanisms of androgen mediated gene expression.

This basic knowledge and technology will then be applied to the human prostate and its abundant messenger RNAs. Potential markers for normal, as well as abnormal prostatic tissue, can then be developed. Abnormal gene expression may be represented by synthesis of sequences homologous to any of the v-oncogenes now under

investigation in a number of laboratories. Prostate disease in humans is of high incidence and has an environmental component. The markers we develop may, therefore, be of some usefulness in a clinical setting.

PUBLICATIONS

Carter, D. B. and Resnick, M.: High-resolution analysis by two-dimensional electrophoresis of human prostatic fluid. The Prostate 3: 27-33, 1982.

Carter, D. B., Newbold, R. R., McLachlan, J. A., and Harris, S. E.: Molecular differentiation of the mouse genital tract: Protein synthesis in fetal and immature female reproductive tract. Biol. of Reprod. (In press).

Carter, D. B., Yamada, K., Monahan, J. J. and Harris, S. E.: Investigation of androgen dependent mRNA from rat ventral prostate using cloned cDNA. J. Biol. Chem. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70060-09 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Development and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. McLachlan	Head, Transplacental Toxicology Group	LRDT	NIEHS
OTHER:	K. S. Korach	Research Endocrinologist	LRDT	NIEHS
	R. R. Newbold	Biologist	LRDT	NIEHS
	G. H. Degen	Visiting Fellow	LRDT	NIEHS
	S. E. Harris	Senior Staff Fellow	LRDT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	T. Eling	Research Pharmacologist	LPFT	NIEHS

COOPERATING UNITS (if any)
Bowman-Gray School of Medicine
Duke University Medical Center
Medical Foundation of Buffalo
University of Würzburg

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Transplacental Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	2.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range goals of this project are: (1) to evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring; (2) to investigate the mechanisms involved in the production of subfertility in mammals as a result of their in utero exposure to foreign chemicals; (3) to assess the transplacental carcinogenic potential of these compounds; (4) to study the physiologic disposition and metabolism of these compounds in the pregnant animal and fetus; (5) to study chemico-biological interactions of transplacental toxicants, with special emphasis on structure-activity relationships; (6) to determine if prenatal exposure to environmental agents can alter the biological response to steroid hormones in reproductive tract tissues; (7) to develop and utilize organ culture systems to study the effects of environmental chemicals on the development of the fetal reproductive tract in vitro; and (8) to evaluate the above animal models as predictors of human response. Special attention is given to diethylstilbestrol (DES).

PROJECT DESCRIPTION

METHODS EMPLOYED: The most sensitive measurement of a female's gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the function status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, and other parameters of function. Pharmacological, biochemical, physiological, and morphological procedures were used which included polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, inverse isotope dilution analysis and chromatography, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS AND PROPOSED COURSE: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock growth promotor and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring. Continuation of these studies has shown that such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma, squamous cell tumors of the vagina and ovarian tumors. A common non-neoplastic lesion of the DES-treated mouse was squamous metaplasia of the uterus. This abnormality was determined to require a secondary stimulus by estrogen at puberty for its manifestation. Anatomical changes such as cervical enlargement without luminal size changes, altered utero-tubal junctions, and uterine shape changes may be important to understanding reported subfertility in similarly exposed women. Alterations in the surface morphology of the DES-treated mouse genital tracts were revealed by scanning electron microscopy. Stromal hyperplasia of the vagina, cervix and uterus has raised the question of the role of this tissue component in the observed lesions; the demonstration of uterine and cervical stromal sarcomas in prenatally DES-treated mice further emphasizes the importance of studies on stromal-epithelial interactions during abnormal development of the genital tract. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β-estradiol. Differential fetal protein binding of DES and estradiol may help explain these results. Bioavailability at many levels may be a determining factor in the transplacental toxicity of hormonally-active xenobiotics. Moreover, the stage of cell differentiation at the time of DES exposure is critical in determining the type of lesion expressed later in life. DES treatment during early cytodifferentiation of the female genital tract is associated with vaginal adenocarcinoma but very little vaginal adenosis; treatment during later cytodifferentiation of this tissue is associated with adenosis but not vaginal adenocarcinoma. Ovarian abnormalities in morphology and steroid secretion in prenatally DES treated mice appear to arise from altered differentiation of the fetal ducts which contribute to ovarian morphogenesis.

In continuation of studies with male offspring from DES treated mice, the fate of Müllerian duct remnants in DES treated males was determined and the role of these tissues in prostatic lesions evaluated. These remnants were shown to respond to estrogen with typical Müllerian features. Müllerian remnants were observed in

treated male offspring at any stage of development. Alterations in these tissues were, in part, related to changes in estradiol and Müllerian inhibiting factor (MIF). Most abnormalities observed in the genital tracts of exposed men and women have been duplicated in our mouse model. Additional alterations seen in the mouse may be predictive for the human. In both male and female offspring, attempts will be made to establish biochemical/morphological markers for genital tract lesions. For example, the SDS protein profile of the secretions of the prostate and seminal vesicles of DES mice were shown to be altered; castrate males exposed prenatally to DES and postnatally to estradiol had seminal vesicle secretory patterns identical to uterine luminal fluid.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g, β-dienestrol and ω-hydroxy DES) were identified in the mouse fetus and neonate exposed to ^{14}C-DES. Recently, organ cultures of the fetal mouse genital tract have been shown to oxidatively metabolize DES. Studies on the bioactivation of DES have shown the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from target tissue, the mouse uterus. DES was shown to be oxidatively metabolized during prostaglandin synthesis by the peroxidase activity of prostaglandin synthetase from sheep seminal vesicle microsomes. The role of this activity in target organ bioactivation remains to be established. The estrogenic activities of a series of DES metabolites and analogs were determined. Results suggest that DES metabolism follows alternative pathways resulting in metabolites which retain estrogenicity or those in which such activity is absent. These studies have been expanded with special emphasis on the biological significance of potentially activated metabolites; such data should aid in generalizations to other classes of estrogenic environmental chemicals. In addition, studies of DES metabolism in target/non-target tissues and in cell culture are being continued. For example, it was shown that peroxidase, an inducible enzyme in estrogen target tissue, is able to metabolize DES to its major metabolite, β-dienestrol. Fluorinated derivatives of DES have been made to help assess the role of metabolism in toxicity. These studies are augmented by experiments on the in vitro metabolism of DES by a transformable cell system in which conversion of DES to β-dienestrol was demonstrated. Further studies suggest that metabolism of DES by these cells is mediated, at least in part, by co-oxidation with prostaglandin synthetase. Moreover, DES and some of its structural analogs neoplastically transform these cells in vitro in the absence of measurable somatic mutation or stimulated cell proliferation. A better correlation was established for the peroxidative metabolism of the compound and cell transformation than its estrogenicity.

Studies in organ culture have shown that DES can alter normal differentiation of the genital tract in vitro. These studies will be continued to evaluate the role of organ/organ and cell/cell interactions in genital tract development. Thus, DES has been shown to alter the action of MIF on the in vitro differentiation of the Müllerian ducts. Two-dimensional gel electrophoretic maps of the protein changes during organogenesis of the female genital tract have been developed to aid in an understanding of the molecular events which determine normal or abnormal differentiation of this system. Also, experiments utilizing the separation and recombination of stroma and epithelium of DES treated fetal reproductive tracts

have been undertaken to determine the role of such tissue interactions in DES induced genital abnormalities. Studies are being done to evaluate the role of cell proliferation in the fetal genital tract by different DES analogs in their transplacental carcinogenic potential.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such in utero drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte or transplacental carcinogenic changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-06 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives of this project are to determine whether DES is metabolized to biologically and hormonally active metabolites; to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins particularly alpha-fetoprotein; to investigate some of the biochemical mechanisms which contribute to effects of prenatal exposure of mice to hormonally active environmental chemicals; to investigate the mechanism of uterine hormonal responsiveness and to determine the molecular locus of transplacental toxicity using structure-function relationships of different environmental chemicals; and to determine biochemical markers for transplacental toxicity. These objectives are approached using refined biochemical techniques of hormone receptors and hormone action. The basic physiological effects on hormone synthesis and hormone levels will be studied using chemical extraction techniques and radioimmunoassays. The carcinogenic nature of hormonally active environmental chemicals will be studied in vivo.

PROJECT DESCRIPTION

METHODS EMPLOYED: Hormonal effects were studied with receptor binding techniques including saturation binding, competition studies, Scatchard plot analysis, sucrose gradient centrifugation, glucose oxidation/utilization, RNA polymerase enzyme activities, extraction of nuclear DNA polymerase enzymes and quantitation of DNA synthesis, polyacrylamide gel electrophoresis, dual isotope labeling, thymidine and steroid autoradiography and protamine sulfate precipitation. Biochemical studies employed spectrophotometric enzyme assays and routine chemical isolation and extraction techniques. Tissue separation and fractionation techniques are utilized to understand differential cell responses.

MAJOR FINDINGS AND PROPOSED COURSE: Prenatal administration of DES results in female offspring of two distinct groups: those with hormonally nonresponsive uteri and those that are hyperstimulated. To understand why some uteri were not hormonally responsive, the concentration of estrogen receptors in these animals was examined. Results have shown that those animals in the nonresponsive group have significantly lower levels of estrogen receptor. In order to distinquish the age at which this difference in receptor level occurs between DES treated and control animals, receptor levels in 1 to 12 month old animals have been measured. These studies suggest that at 1 to 3 months of age there is no pattern of receptor differences. By 4 months differences in receptor levels between control and treated groups are noticeable and by 6 months significantly lower levels are seen in the DES group. Endocrine manipulation using adrenalectomy can result in an animal with similarly lower uterine receptor levels as the DES animal. However, hormone responsiveness in these animals is not compromised as it is in DES exposed animals. Studies to determine receptor levels in fetal reproductive organs are being planned; a micro steroid receptor assay and estrogen receptor antibody assay will be developed. Cytosol receptor concentrations in vaginal tissue were not significantly different from controls. Further experiments with the uterus and vagina from DES exposed mice will determine which step in the mechanism of hormone action is altered. The differences in cytosol receptor levels seen after 4-6 months could not be explained by differential accumulation of receptors in the nucleus since assays of nuclear receptor in these same tissues showed no appreciable differences.

Receptor differences in control and DES treated offspring were also found in studies demonstrating the responsiveness of the receptors to estrogen administration. The mouse uterus possesses a second translocation of hormone receptor complex to the nucleus after exposure to hormone. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear peak. Steroid autoradiography techniques were used to demonstrate that the two events are occurring in different uterine cell types. There is a temporal pattern of interaction with the hormone appearing in the nuclei of stromal and glandular epithelial cells and later in luminal epithelium. The mechanism for this differential interaction is being investigated in more detail. The role of this event in estrogen action in the mouse reproductive tract, with regard particularly to the actions of hormonally active environmental chemicals, is being studied since DES treated animals appear to have an altered pattern of receptor depletion/replenishment. Receptor synthesis,

induction of progesterone receptor, RNA ploymerase activities, DNA polymerase activities and DNA synthesis are also being investigated. A molecular marker for estrogenic activity in uterine tissue is being sought to determine the activity and mechanism of action of hormonally active chemicals. Protein labeling experiments using [^{35}S] methionine have illustrated several proteins (32,000 - 54,000 mw range) in uterine tissue from estrogenized animals. Nonenzymatic separation of the three uterine tissue compartments have indicated that some of these proteins are unique to one cell type. Proteins from the epithelial compartment show significant isoelectric charge trails suggesting glycoprotein structure. A 79,000 mw protein with multiple isoelectric points was also found in the incubation media. Incubations with uteri from control animals did not show the presence of these proteins. Studies are underway using labeled galactose and fucose reagents as well as neuraminidase digestion to determine the glycoprotein nature of these intracellular and secreted estrogen responsive proteins. Attempts will be made to produce a poly (A^+) mRNA preparation and library to study the mechanism of their synthesis.

In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo bioassay and an in vitro receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity while certain metabolites, e.g. β-dienestrol or ω-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation. The exception to these results were some indenestrol isomers and ψ-DES, which are possible DES metabolites and which show receptor binding comparable to DES, but were 20-100 times less biologically active. These studies have suggested a mechanism of altered/differential clearance of these analog receptor complexes from the target cell nucleus.

Studies of receptor and plasma binding activities, particularly to alpha fetoprotein, of various DES analogs and metabolites will be continued to determine the structural site of chemicals exhibiting hormonal and/or carcinogenic actions. These structural requirements were exemplified by studies determining estrogen mitogenic activity of the DES compounds. Two of the indenestrol isomers, differing only in the position of a double bond, showed divergent uterine DNA stimulation. Another series of ψ-DES isomers has indicated that receptor binding of these ligands can be diminished by subtle structural differences. A unique DES derivative containing fluorine atoms has been synthesized and its hormonal activity is being tested. Preliminary studies indicate this compound interacts with receptor binding sites in a biphasic mechanism and that this complex can translocate to uterine cell nuclei. Tests with this compound and other structural isomers using additional biochemical assays are underway.

Binding studies are being expanded to allow the potential hormonal activity of selected environmental chemicals to be determined using this model. Complete hormone action involves the ability of the hormone to influence synthesis of its receptor; only some of these DES compounds showed this hormonal property which

was related to their biological efficacy. Additional studies have determined that certain DES compounds (e.g. Indenestrol and Ψ-DES) do not significantly stimulate progesterone receptor synthesis. This result may be, in part, the reason for their poor estrogenicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The association of in utero DES exposure and reproductive tract cancer in human beings is well documented. Recent development of a mouse model to study these effects will allow this problem to be more fully investigated. The objectives of this project are to define the roles of receptor protein-chemical interactions and the biochemical mechanisms associated with the toxicologic responses observed in the reproductive tract following in utero exposure to hormonally active environmental chemicals.

Since knowledge of gestational effects of environmental chemicals on the reproductive system of the offspring is so limited, these studies will help identify other clinical and biomedical problems which may arise from exposure to environmental compounds. Determining the mechanism by which these chemicals act will help in the development of reasonable safeguards.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70080-09 LRDT

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October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.2	0.1

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies seek to assess the effects of environmental agents on spermatogenesis, function of accessory sex organs, and male and female reproductive capacity. Mechanisms of toxicity are studied and new approaches to toxicity testing are proposed and validated in order to extrapolate more reliably from laboratory animals to man and to improve our ability to analyze risk. The following studies are ongoing: (A) the mechanisms by which DNA-damaging agents increase the induction of aryl hydrocarbon hydroxylase activity by TCDD in the prostate glands; (B) the effects of TCDD on preovulatory oocytes and preimplantation embryos; (C) protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors; (D) correlation of improved histopathologic assessment (ultra-thin sections) of testicular damage induced by antineoplastic agents with testicular and epididymal sperm counts and fertilizing capacity determined in vivo; and (E) susceptibility of testicular tissues to early postnatal treatment with antineoplastic agents.

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) Chemicals modifying the induction of polycyclic aromatic hydrocarbon activating enzymes. BP hydroxylase (AHH) was measured in prostate and liver of male Sprague-Dawley rats after treatment with TCDD or TCDD plus agents which damage DNA (procarbazine, methylmethane sulfonate, or triethylene-melamine (TEM)). The DNA damaging agents were administered intraperitoneally 24 hours prior to oral treatment with TCDD (10 µg/kg). Hepatic and prostatic microsomes were prepared by homogenizing tissue in 4 volumes of ice cold 0.15 KCl in 0.02 M HEPES, pH 7.4. The homogenate was centrifuged for 15 minutes at 9,000 x g at 4°C. The supernatant was then removed and centrifuged at 176,000 x g for 45 minutes to obtain microsomes and soluble fraction. AHH activity was assayed by measuring the fluorescence of alkali-extracted BP phenols with a Aminco Bowman spectrofluorometer using 3-OH BP as a standard. The liver and prostatic total RNA was extracted by the guanidine HCl procedure, followed by oligo(dT)-cellulose chromotography twice and precipitation in ethanol. The poly(A^+)-enriched RNA was electrophoresed on 1% agarose gel containing 10nM methylmercury hydroxide and then adsorbed to DBM paper. The mRNA on the DBM paper was prehybridized for 2 hr at 68°C with 5X SSC, containing 2X Denhardt's solution, salmon sperm DNA (250 µg/ml), and 0.05% SDS. The mRNA on the filters was then hybridized overnight at 68°C in the prehybridization buffer with 1 x 10^7 dpm of ^{32}P-DNA from pAhP-2.9. The DBM papers were washed with 0.1x SSC containing 0.05% SDS at 52°C and exposed to Kodak RP-5 x-ray film at -70°C in cassettes equipped with intensifier screens. 23S and 20S mRNA in liver and 23S and 21S mRNA in prostate glands were cut out from the paper and the respective ^{32}P radioactivities were quantitated.

(B) Correlation of morphology and protein patterns in TCDD treated preimplantation mouse embryos. Preimplantation embryos from mice were collected at different stages of development. Early stages, still surrounded by cumulus cells (both unfertilized and 1 cell stage), were isolated by puncturing the oviduct with fine needles. The cumulus cells were then dissociated with hyaluronidase treatment. Later stages (2 cell to blastocyst stages) were flushed from the oviducts and uteri, respectively. The embryos were then cultured. Embryonic protein synthesis at various stages was determined by culturing the embryos in a culture media containing ^{35}S-methionine (2 mCi/ml) with 5%CO_2, 5%O_2, and 90%N_2 for 2-5 hours. The newly synthesized proteins were extracted and solubilized in SDS-urea-DTT and NP-40 ampholine or in Lammeli buffer. Two-dimensional gel electrophoresis was carried out according to the O'Farell's method. Following the gel electrophoresis, the SDS-polyacrylamide gels were dried and allowed to expose x-ray films. The patterns of newly synthesized proteins at various embryonal stages were examined. Preimplantation mouse embryos from 1 cell to blastocyst stages were collected at different time intervals after mating untreated males with TCDD-treated females. The embryos collected were cultured in vitro and their morphological changes were observed under phase microscopy. Abnormal development at various stages of embryo development was scored.

(C) Protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors. Epithelial cells from precaput, caput, corpus, and cauda epididymides were isolated by microdissection methods developed in our laboratory. Precaput, caput, corpus, and cauda epididymides

were dissected, under a stereomicroscope at 60x magnification, with a micro-scissor. The connective tissues and septa were removed and tubules from each section of the epididymides were freed into a Petri dish. Epididymal tubules were sectioned into approximately 1 mm lengths and sperm flushed out by gentle pasteur pipetting. Tubular fragments were then washed with PBS three to four times to remove sperm contamination. Subsequently, proteins from the isolated tubules were separated using SDS-polyacrylamide gels. Two-dimensional gels were stained with either Comassie blue or silver stain. Differences in the protein patterns of various parts of the rat epididymides were compared.

(D) Correlation of improved histopathologic assessment (semi-thin sections) of testicular damage induced by antineoplastic agents with testicular and epididymal sperm counts and fertilizing capacity determined in vivo. A recently developed histological approach involving organ perfusion, plastic embedding, and semi-thin sectioning, has been used to assess testicular damage induced by various anti-cancer drugs selected for their mode of action. Morphological findings are being correlated with alterations in sperm counts and disturbances in male reproductive function determined by serial mating. Sprague-Dawley male rats, 10 weeks of age, were treated once i.p. with the test agent. Studies of procarbazine, 50 and 200 mg/kg are discussed in this report. To properly fix the tissues, the testes were perfused initially with physiologic saline to wash out the blood, followed by 5% glutaraldehyde in 0.2 molar cacodylate buffer (pH 7.4) after about one minute. The hardened tissues were cut into small blocks and postfixed for 90 minutes in 1% osmium tetroxide and 1.5% potassium ferrocyanide to enhance contrast. The flat embedded specimens were cut at 1 μ thickness. Sperm were quantified by counting sperm heads in the testis and epididymides. Fertility was assessed by serial mating.

(E) Susceptibility of testicular tissues to early postnatal treatment with anti-neoplastic agents. Unique susceptibility to chemical toxicity is critical to defining hazards and analyzing risks. Testicular development, because it involves both pre- and postnatal periods and includes the differentiation of various tissues, offers a number of possible targets for chemicals capable of perturbing biological processes. Thus, we undertook to determine whether anticancer agents, selected for their mechanisms of action, could probe differential toxic effects of spermatogenic, Sertoli and/or Leydig cells if administered acutely on selected postnatal days. Male Sprague-Dawley rats were treated i.p. once with well tolerated doses of anticancer drugs (cyclophosphamide-C, cytosine arabinoside-CA, vinicristine-V, procarbazine-P, or doxorubicine-D) on either postnatal day 6, 16, 24 or 45. Morphological observations were correlated with testicular and epididymal sperm counts, time of puberty, and male reproductive capacity.

MAJOR FINDINGS AND PROPOSED COURSE: (A) Chemicals modifying the induction of polycyclic aromatic hydrocarbon activating enzymes. Oral TCDD pretreatment results in a 200 fold increase in prostatic AHH activity. This induction is potentiated 5 fold (1000 times control level) by prior intraperitoneal treatment with DNA damaging agents. Following either TCDD or TCDD plus DNA damaging agents, P_1-450 mRNA, as measured by hybridization to a ^{32}P-DNA probe from pAhP-2.9, increased several folds. In liver and prostate glands, mRNA of two different

sizes were found. In prostate glands, 23S and 21S mRNA were found to hybridize with a subclone of pAhP-2.9 DNA. Prostatic P_1-450 mRNA levels 24 hrs after either TCDD alone or TCDD plus alkylating agent were 18 and 30 times that of control, respectively, and thus, reflected well the magnitude of AHH induction. 23S mRNA in control, TCDD, and TCDD plus procarbazine treated animals were 2 fold greater than that of 21S mRNA. Further studies are needed to fully understand the mechanisms of potentiation of TCDD-induction of AHH activity by DNA damaging agents. Whether DNA damaging agents expose a greater number of TCDD binding at the Ah locus in the genomic DNA needs to be elucidated.

(B) Correlation of morphology and protein synthetic patterns in TCDD-treated preimplantation mouse embryos. Female CD-1 mice treated with a single intraperitoneal dose of TCDD (0, 12.5, 25, and 50 µg/kg) were mated with an untreated male. Effects of TCDD on oocytes and subsequent embryonic development through 1, 2, 4, and 8 cell, morula and blastocyst stages were evaluated using in vitro embryo culture. A significant number of embryos at the morula and blastocyst stages were affected by TCDD treatment 72 hrs prior to the superovulation and mating. Fragmented and collapsed embryos at morula and blastocyst stages were common. The results suggest that high levels of embryo toxicity in vivo are due to both direct toxic effect on pre-ovulatory oocytes as well as on the conceptus. The exact mechanism of TCDD associated cytotoxicity on either pre-ovulatory cocytes or on embryos is not known.

Studying normal embryonic development, marked differences were found in the relative amount of newly synthesized protein at the different preimplantation stages. Two and four cell stages showed methionine incorporation of 620 and 1,200 dpm per embryo or 24,000 and 56,000 dpm per mg protein, respectively. In contrast during the morula and blastula stages, incorporation of methionine was 5,000 and 6,060 dpm per embryo, or 238,000 and 202,000 dpm per mg protein, respectively. Therefore, the protein synthesis during the blastula stage appears to be about 4 times that of the 4-cell stage. Two-dimensional gel electrophoretic patterns are being utilized to evaluate effects of environmental chemicals on female (or male) germ cells. Altered protein synthesis at various preimplantation stages are being studied.

(C) Protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify maturation factors. Two-dimensional protein patterns of epithelial cells from precaput and caput compared to cauda epididymis were strikingly different. Because these differences in the protein patterns were reproducible, they might serve as markers for detecting alterations in epididymal cell function with respect to sperm maturation and determine sperm specific surface proteins of epididymal origin.

(D) Correlation of improved histopathological assessment (semi-thin sections) of testicular damage induced by antineoplastic agents with testicular and epididymal sperm counts and fertilizing capacity determined in vivo. Procarbazine treatment was relatively well-tolerated by rats and organ toxicity was seen only in the testes. Testicular weights were significantly ($p < 0.05$) decreased following both doses to a minimum four weeks after treatment. Reduction of epididymal weight was less pronounced. Slight effects on the germinal epithelium were observed three

days after treatment which progressed rapidly to a maximum after four weeks when practically all tubules were affected. The first Sertoli cell-only tubules were found at this time. The germinal epithelium recovered gradually during the following weeks until 10 weeks after treatment when the only major findings were a small percentage of atrophic tubules. Early subcellular changes, especially in early spermatids, included rounding of the chromatoid bodies, indentation of the nuclei by acrosomal granules and the tendency of nuclei to round up and become very condensed. Furthermore, the acrosomal development seemed to be retarded which led to a desynchronization between early spermatids and the other cell types, especially in the late spermatids.

Following procarbazine treatment, a decrease in fertility was observed in each mating period during the ten week trial. In controls, 67 out of 70 mating resulted in litters with more than four viable implants, whereas only 56 out of 70 matings were successful in the treated group. Fertility was significantly reduced compared to the control values during weeks 9 and 10. This fertility profile suggests that the spermatogonia, late spermatocytes, and early spermatids are the most susceptible to the actions of procarbazine. The number of normal litters produced by the males was 65 for the control and only 40 for the treated group. Values observed for treated animals are less than control at each week and are significantly reduced in weeks 2, 9, and 10. Spermatogonia, late spermatocytes, and early spermatids seem to be most susceptible to this effect.

Sperm counts in the testes were decreased two weeks after treatment and remained low until about the seventh week after treatment when recovery was evident. The correlation was good between the testicular sperm counts and the morphological changes observed in the seminiferous epithelium.

(E) Susceptibility of testicular tissues to early postnatal treatment with antineoplastic agents. Unique tissue and cellular susceptibility was observed when anticancer agents were administered to rats at selected postnatal periods. Sertoli and Leydig cells replicate postnatally only early in life, and the cell populations are stable thereafter. Spermatogenesis is initiated shortly after birth. When male Sprague-Dawley rats were treated i.p. once with doses of anticancer drugs on either postnatal day 6, 16, 24, or 45, the following effects were observed: V (all 4 treatment days) and C (day 16, 24, 45) delay puberty; C (16, 45), CA (16, 24, 45), and V (6) increase reabsorptions; V (45) reduces sperm counts; and that C (16) and V (16, 24, 45) cause sterility in some of the animals. Reduced epididymal weight is found with C (16, 24) and V (24, 45). Histologic evaluations suggest an association between damage to a particular developing cell type and an observed dysfunction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Toxicological studies of a target organ, such as the testis, ovary, or male accessory glands, seek to elucidate qualitatively and quantitatively the toxic effects of a chemical on that organ. The ultimate objective is to assess the toxic effects of the chemical on laboratory animals and extrapolate pertinent experimental data to man. To accomplish these objectives, one must consider the main factors which may influence and modulate the toxic effects of chemicals in those organs. In the male gonads and accessory glands, such modifying factors are the pharmacokinetic

parameters governing the absorption, distribution, activation, and deactivation of toxicants; covalent bindings to macromolecules; DNA damage as well as DNA repair of damaged germ cells and accessory glands, All of these factors are being studied in our laboratory at the present time.

Short-term tests of reproductive and developmental toxicity are also sought. A better understanding of the epididymal sperm maturation processes is important with respect to reproductive biology and also provides a better insight into how exogenous chemicals may exert their effect on epididymal sperm maturation. Chemicals affecting this sperm maturation process might also suggest new approaches to fertility control.

Modern histological techniques (semi-thin sections) have been used to correlate morphological changes with physiologic (sperm counts) and functional parameters (fertility). Cytotoxicity and malformed germ cells are relatively easy to recognize morphologically, especially with these newer histological techniques. However, it is not possible to determine with these techniques whether effects are primarily of a nongenetic or genetic nature. Only drastically reduced sperm counts lead to a decrease in fertility. Increased early pregnancy loss and abnormal development of implanted conceptuses observed during in vivo studies suggest genetic toxicity. Thus, it appears that even in a case of potent chemicals, a battery of different approaches including morphology, sperm counts, and serial mating are necessary to evaluate the complete spectrum of toxic actions which affect male fertility.

Because the testicular compartment is populated by various cell types which differentiate and replicate during specific postnatal periods, these cells are particularly susceptible (or resistent) to damage by antineoplastic agents selected for their mechanisms of action. Most anticancer agents affect dividing cells and therefore their therapeutic effectiveness is dependent on cell turnover rates and treatment schedule. In a similar way, treatment schedule should be able to target Sertoli, Leydig, or spermatogenic cells. The possibility exists that with carefully selected doses and treatment regimens, laboratory animals could be produced which are deficient in one of these cell types. Such animals would be valuable models, especially to further explore the physiological role of the Sertoli cells.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70085-05 LRDT

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of In Vitro Models for Assessing Reproductive Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. P. Lee	Pharmacologist	LRDT	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LRDT	NIEHS
	R. Bechter	Visiting Fellow	LRDT	NIEHS
	M. Matsuda	Visiting Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Because both laboratory animals and the commonly used parameters for semen analysis are unreliable predictors of altered human male reproductive capacity, various test approaches utilizing sperm are being developed and validated. Attention is currently directed to three different approaches: (1) interspecies (human sperm/hamster ova) in vitro fertilization; (2) plant lectin interactions with heterogenous surface glycoprotein binding sites during sperm maturation; and (3) analysis of heterogenously distributed sperm surface proteins appearing during sperm maturation with monoclonal antibodies. Interspecies in vitro fertilization test results were compared with pregnancies and other clinical data of couples consulting a fertility clinic and initial results demonstrated that the test was a valid predictor of altered fertility. Research findings regarding the other two approaches are still preliminary.

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) Interspecies in vitro fertilization. Eighty infertile couples attending a fertility clinic agreed to participate in the study. A standard infertility evaluation was performed on each couple. A group of 30 normal men who had fathered children also participated in the study. Semen for each male was evaluated for volume, liquification, viscosity, color, pH, total sperm count, concentration (sperm density), progressive motility at 1 hr, motile sperm count, percent dead and percent abnormal forms. Sperm were prepared so that each fertilizing dish contained approximately equivalent numbers of motile sperm. Ova for fertilization were collected from Golden hamster oviducts by superovulation; they were denuded and incubated in vitro with sperm suspensions from either fertile or suspected infertile men. After three hours, ova were examined by phase-contrast microscopy for morphological evidence of fertilization. In order to determine the validity of this in vitro test, pregnancies for participants in the study are also being followed.

(B) Lectins interact with specific glycoproteins on cell surface membranes and may be useful probes to monitor alterations in the number, distribution, and mobility of cell surface receptors associated with cell maturation and with cell-cell interactions. Therefore, various lectins were employed to determine modifications in rat sperm plasma membrane during testicular and epididymal maturation processes. Fluorescein-conjugated concanavalin A (Con A), wheat germ agglutinin (WGA), soybean agglutinin (SA), Ricinus communis 120 (RA), and Ulex europeus (UE) were employed to determine changes in the sperm surface membrane during the sperm maturation processes. Spermatozoa from testis, caput, and cauda epididymis and vas deferens were washed twice with phosphate buffered saline (pH 7.4). An equal number (10^8/ml) of washed spermatozoa from testes, caput, and cauda epididymides and vas deferens were incubated with the selected lectins at equimolar concentrations (0.1-0.4 mg/ml) at room temperature for 30 min. After incubation, spermatozoa suspensions were microscopically examined, and fluorescence visually quantitated on a scale from 0 to 5+.

(C) Monoclonal antibodies derived from hybrid cell lines provide highly specific probes that recognize individual determinants. Monoclonal antibodies to sperm surface components can be used to determine sperm surface protein alteration during sperm maturation or chemically-induced toxicity. To obtain specific monoclonal antibodies against spermatozoa (SPZ) surface proteins at specific stages of maturation, SPZ were obtained from the precaput, caput, and cauda epididymides of the Fisher 344 rat, age 12 weeks. The SPZ were suspended in 40 ml phosphate buffered saline (PBS) and were washed twice by centrifugation at 250g. DBA/2 male mice, 8-12 weeks of age, were first inoculated subcutaneously with 5×10^7 SPZ in Freund's complete adjuvant. Male mice were subsequently immunized with 1×10^7 SPZ administered i.p. on days 21 and 28 prior to isolation of splenocytes. Splenocytes were obtained from minced spleen tissue and fused with myeloma cells. Suspended in RPMI 1640 medium with 20% fetal calf serum (FCS), 10^8 splenocytes were mixed with 5×10^7 myeloma cells (TIB-18) and centrifuged at 500g for 7 min. Polyethylene glycol was added dropwise (0.8 ml) and one min later, 20 ml of FCS free RPMI 1640 was added with gentle mixing and centrifuged.

The pellet was resuspended in RPMI 1640 with 20% FCS and 1.0 ml placed in each well of a 96 well culture plate. The cultures were maintained in HAT selection medium for 14 days at 37° C (95% O_2 and 5% CO_2). Three weeks after fusion, the hybridoma supernatant was screened for relevant antibodies using an enzyme linked immunoabsorbant assay or FITC-conjugated rabbit anti-mouse IgG and IgM. Positive wells were split and cell lines from positive hybrid cells cloned further.

MAJOR FINDINGS AND PROPOSED COURSE: (A) For years it was accepted that male infertility was the result of a deficiency in sperm motility, sperm number, normal sperm morphology or a combination of semen inadequacies. Recent findings in this study, however, demonstrated that a deficiency in an intrinsic factor necessary for sperm fertilizing capacity may exist even when all or most of the standard parameters are normal. Since the conventional semen analysis currently used for predicting male fertility is not totally reliable, the correlation of interspecies in vitro fertilization results (based on the penetration of zona pellucida-free hamster ova by human sperm) with clinical evaluation of the infertile couple was investigated. In vitro test results for 154 males evaluated correlated well with clinical diagnosis. Sperm from one-fifth of the patients penetrated no hamster eggs at all, a phenomenon not observed in the normal donor population. Yet, half of those patients whose sperm fertilized no hamster ova in vitro had normal semen parameters. No subsequent pregnancies have occurred in this group. In contrast, 21 of 23 couples achieving pregnancies or delivering during the study gave positive fertilization results in the in vitro test-system. Although one cannot morphologically distinguish between "fertile" and "infertile" sperm, the validity of this test suggest that it can accurately assess the fertilization potential of a particular patient subpopulation without having to wait on the success of pregnancy. Further studies will seek to determine the subtle biochemical (or cellular) deficit in sperm from infertile men with normal sperm parameters.

(B) During sperm passage from the testes, through the caput and cauda epididymides and vas deferens, binding of Con A, WGA, RA, and UE increased with increasing maturity of sperm. In contrast, SA binding was greatest in testicular sperm and SA binding decreased with increasing maturity. Fluorescein-conjugated lectins are found associated with acrosomal, postacrosomal, neck, mid piece and along the tails of sperm. These findings suggest that as the sperm mature, passing through various regions of the epididymides, glycoproteins containing D-glucose, D-mannose, N-acetyl-D-glycosamine, L-fucose, and D-galactose increased significantly. In contrast, the N-acetyl-D-galactosamine binding site for SA was greatest in the testis, but decreased with increasing maturity of spermatozoa. The order of lectin binding sites for spermatozoa from vas deferens was Con A > UE > RA > WGA > SA, respectively. These findings suggest D-glucose and D-mannose in sperm surface glycoproteins increased as sperm matures more than other carbohydrates.

(C) Because lectin binding to sperm at various stages of maturation was not as specific or quantitative as one would like, the analysis of the sperm surface protein at the different stages of maturation with monoclonal antibodies was undertaken. Hybridoma cells are being produced by fusing myeloma cells with splenocytes from male mice immunized with sperm from precaput, caput, and cauda

epididymides of F344 male rats. Clonally derived cell lines will be selected which produce monoclonal antibodies against sperm surface components. Each of the sperm monoclonal antibodies bound to a specific region of the sperm surface, as seen by indirect immunofluorescence, will be quantitated to define sperm maturation factors. Following these developmental studies, similar techniques will be used to study changes in sperm surface proteins induced by environmental chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: More specific tests of sperm functionality are needed to improve the reliability of currently used clinical tests. The human hamster interspecies in vitro fertilization model applied at the NIEHS provides a useful tool to better evaluate the relationship between human reproductive health and the chemicals in our environment. These test-systems which can utilize human sperm will increase our capabilities to analyze sperm function. The application of monoclonal antibodies against heterogenous sperm surface protein is especially exciting. These antibodies may produce valuable probes for determining altered sperm surface proteins which can be correlated with the capacity of sperm to fertilize ova in vitro and in vivo.

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COMPARATIVE MEDICINE BRANCH

COMPARATIVE MEDICINE BRANCH
Summary Statement

The Comparative Medicine Branch (CMB) of the National Institute of Environmental Health Sciences programs and coordinates experimental animal procurement, housing and utilization for the Institute; advises Institute scientists of appropriate animal models for use in research programs; maintains a laboratory for the diagnosis and research of animal diseases; operates a rodent genetics resource, including gnotobiotic capability; operates glassware and media kitchens serving the Institute; and investigates and implements new or improved methods of achieving these missions. The Comparative Medicine Branch consists of four sections including Animal Husbandry, Diagnostic & Research Laboratory, Quality Assurance, and Glassware and Media.

ANIMAL HUSBANDRY

Animal Husbandry is responsible for the procurement and maintenance of experimental animals and technical assistance to investigators using animals in their research program. AH investigates and tests new systems aimed at two specific objectives: (1) improved efficiency operation, and (2) elimination of adventitious influences on animal experimentation. Currently AH is conducting experiments in collaboration with other Institute laboratories to investigate the influence of ventilated caging systems on metabolism baselines as a preliminary study to determine if movable ventilated caging systems are acceptable as alternatives to hard site toxicology containment units, thus enhancing the Institute's ability to conduct toxicology studies. The second program is aimed at preventing infectious disease spread in small rodent colonies and is centered upon improved methods of containment and sanitation. Presently the entire small rodent colony is under filter caps and surveillance programs indicate some reduction in the distribution of infectious diseases; however, the program at this point is only six months old and further experience and testing will be necessary to evaluate the system. A third program centers on the study of a new logistics scheme for handling materiel in the animal facility to improve and expedite the flow of equipment and the level of sanitation. This system will make use of new types of materiel handling equipment as well as modify the pattern of personnel work effort. The fourth effort is aimed at improving the pasteurization/sterilization technique for animal feed at the Institute. Specifically the effort is directed in reducing the heat exposure time for feed in order to reduce the harmful effect of heat on labile nutrients. These studies indicate that a cycle which will result in total chamber exposure time of approximately 30 minutes vs one hour and thirty minutes is entirely feasible.

During the past year AH has established and brought on-line a rodent genetics resource located at the Duke University Farm site and instituted a gnotobiotics capability in order to facilitate the acquisition and propagation of special strains of rodents. The facility is now operational; however, staffing problems at the supervisory level must be resolved. The facility also has limited capability for routine production of special rodent populations; i.e., the nude mouse, and the Fischer-344/Bd rat, which are important to Institute programs but either unavailable or available only at irregular intervals from outside sources. In addition to this facility, AH will bring on-line in August a remote isolation

facility intended to further enhance the ability of Institute investigators to acquire exotic rodents and begin research protocols with these animals prior to complete health status assessment by CMB.

DIAGNOSTIC & RESEARCH LABORATORY

The Diagnostic & Research Laboratory is responsible for health surveillance of Institute animals, health monitoring of supplier colonies of animals, and targeted research in problems of particular interest to CMB and the Institute. DRL is responsible for establishing and testing new methods of diagnosis of animal disease. Current efforts center upon methods which will provide ready answers to the health status of exotic animals. These include methods in cohabitation, mouse antibody production, cortisone stressing, neonate inoculation, and virus serology methods. Important questions targeted for study include the epidemiology of mouse hepatitis virus, currently found in Institute mouse colonies and known to be detrimental to research programs. In close collaboration with the Animal Husbandry section, DRL is studying the effect of modified sanitation and animal handling techniques in the animal rooms as well as conducting the examinations necessary for health profile assessment in the animal facility. A second targeted objective of DRL research is the establishment of the duration of inapparent shedder status for mouse hepatitis virus infected mice. Current information suggests that inapparent shedder status may persist for a long period of time; however, preliminary experiments suggest that this may not be true or may only be true under certain circumstances. Further studies are currently in progress.

DRL is also interested in the relationship of rabbit coronaviruses identified in the USA to those identified in Scandinavia. Experiments are planned to determine if the two reported infections are the same and if so, what is the significance of the discovery with particular reference to its potential impact on research using lagomorphs.

DRL routinely conducts necropsies on dead animals and those ill enough to be recommended for sacrifice. DRL personnel in collaboration with other CMB staff members closely advise investigator staff on potential pitfalls from infectious diseases and methods to prevent or reduce the impact on their experimental studies.

Current efforts are also underway to apply computerized data retrieval to handle and retrieve data generated by health surveillance and monitoring programs. DRL is responsible for maintaining close contact with similar diagnostic laboratories around the country and to take advantage of data generated by those laboratories.

QUALITY ASSURANCE

This is a new program intended to interface closely with DRL, AH, and Glassware and Media to maximize the important quality assurance requirements pertaining to quality of feed, water, animal health, sanitation procedures, sterilization and pasteurization techniques, and media preparation. This section is responsible for establishing toxic substances monitoring schedules for feed and water, as well as acceptable limits for the items tested. Because of the Institute's primary obligation to investigate environmental factors influencing health, it is essential to Institute programs that the potential for influence by environmental

factors such as toxic substances on experiments be minimized and their levels clearly documented for scientific records. Important examples include: levels of nitrosamines present in animal feed, levels of estrogen-like substances present in animal feed, levels of halomethanes present in drinking water, etc. Efforts to generate baseline data will influence management decisions on the implementation of control measures, notably with feed acquisition, pasteurization, storage and water treatment. The QA program extends to close collaboration with DRL in establishing standard methods for testing, and types of tests to be conducted. It is expected that the QA program will guide the Glassware & Media Kitchen through problems relating to media contamination, sterilization, and sanitation. This program is on a trial basis and will be reassessed at a later date.

GLASSWARE & MEDIA

This section provides over 95% of all media and biological fluids used at the Institute, including the preparation of approximately 300 different formulations. This section is responsible for all glassware and sterilization as well as pickup and delivery. The GW&M operation handles over 174,000 pieces of glassware items per month and prepares over 2,500 liters of media per month. During the Winter of 1981 and Spring of 1982 staffing levels were depleted to the point where GW&M services were extended to the limit. In addition, the growth of certain programs requiring large numbers and large varieties of media led to very tight work schedules and improved ordering systems. GW&M's management effort is currently being directed at improved methods of operation, the addition of equipment to improve efficiency, and the cross-training of personnel to reduce the possibility of critical area shortages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 22100-01 CMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Microbial, Nutritional, and Chemical Analyses of NIEHS Rodent Diets: Significance to Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. E. Thigpen Microbiologist CMB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section, CMB

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic & Research Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.2	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three shipments of feed and two water samples (pre- and post-distillation) have been analyzed for microbial and chemical contaminants. All three shipments of feed were accepted. Results were within the acceptable range for the chemical contaminants (heavy metals, chlorinated hydrocarbons, PCB's, BHC's, organophosphates and nitrosamines) and the results were negative for estrogenic activity by the mouse bioassay test.

The level of microbial and chemical contaminants in the tap water was less than the EPA contaminant levels published for potable water. The Vaponic still was effective in reducing the fluorine, nitrate, and total trihalomethanes levels in the tap water. However, we were not able to measure the effectiveness of distillation on all the chemicals listed because pre- and post- results were the same, indicating the sensitivity of the method used was too low or that distillation did not reduce them any further.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Each shipment of NIH-31 feed for NIEHS rodents will be milled at least 2-3 weeks in advance to allow adequate time to determine the microbial, nutritional and chemical quality of the diet prior to consumption.

Seasonal water samples (pre-tap and post-vaponics distillation) from the RGR and from the still in Building 15 will be analyzed to determine the effectiveness of the Vaponics still in removing microbial and chemical contaminants. The microbial analysis of the feed and water will be performed by the DRL. A contract will be awarded for the chemical analyses of feed and water. The work proposal will consist of four parts: 1) Nutritional and chemical analyses of feed; 2) Analysis of feed for nitrosamines - NDMA, NDEA, NDPA, NDBA, NPIP, NPYR, and NMOR; 3) Analysis of feed for estrogenic activity (mouse bioassay); 4) Analysis of water for chemical contaminants.

MAJOR FINDINGS AND PROPOSED COURSE: Three different shipments of feed, milling dates 2/9/82, 2/24/82, and 4/1/82, have been analyzed for nutritional and chemical quality. In brief, all three shipments were accepted. These shipments were within the acceptable range for chemical contaminants and were negative for estrogenic activity. The fat content in the 4/1/82 shipment was low (3.4%).

Only two water samples have been tested. The level of microbial and chemical contaminants in the tap water was less than the EPA contaminant levels stated for potable water. The Vaponic still was effective in reducing the fluorine (1.0 to less than 0.1 mg/liter), nitrate (0.47 to less than 0.01 mg/liter), and the total trihalomethanes (72.0 to less than 1.0 mg/l), and especially chloroform (65.0 to less than 1.0 mg/l) levels in the tap water. However, we were not able to measure the effectiveness of distillation on some chemicals tested because the results were the same for pre- and post-distillation, indicating the sensitivity of the method used was too low or that distillation did not reduce them any further. The microbial quality of both the feed and water was acceptable.

In the future feed shipments will be received every two months (instead of monthly), thus reducing our testing cost. We will proceed as planned for this year and then re-evaluate our quality control program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The principal activity at the Institute involves biomedical research on the effects of environmental agents on human health. Most studies involve chemicals and the use of laboratory animals. Therefore, it is essential that the animal diets (both feed and water) be free of microbial and chemical contaminants and that the diets meet specified nutritive levels. The use of feed or water contaminated with pesticides, nitrosamines, estrogenic compounds, etc., or feed with too low or too high nutritive levels could have a profound influence on the research at the Institute. Thus, it is imperative that we monitor the diets in an effort to detect and avoid these problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 22101-01 CMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Prevalence of Adventitious Infectious Viruses in Research Colonies: Significance to Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Thigpen	Microbiologist	CMB NIEHS
OTHER:	J. D. Small	Veterinary Medical Officer	CMB NIEHS
	C. B. Richter	Chief, Comparative Medicine Branch	CMB NIEHS

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.5	1.0

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[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

From October 1, 1981, to the present, a total of 2,013 serological tests were performed on 462 sera. Included were 153 rat sera (660 tests), 271 mouse sera (1,225 tests) and 38 hamster sera (128 tests). Health surveillance data from NIEHS breeding colonies and from experimental animals revealed that: 1) Four different rat viruses (Sendai, PVM, KRV, and RCV/SDA), 2) Three different hamster viruses (Sendai, PVM, and SV-5), and 3) Four different mouse viruses (Sendai, PVM, MHV, and Reo-3) have been detected in animals housed in NIEHS facilities. These data suggests that MHV is widespread in our mouse rooms and that some incoming mice had seroconverted to MHV positive within 3 weeks after arrival. These mice were housed under filter tops which should limit or reduce aerosol transmission. Studies will evaluate factors which affect the distribution of infectious MHV among animals. Specifically, we will attempt to demonstrate the length of the inapparent carrier state of experimentally infected mice.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Serological methods (HAI, IF, CF, and the ELISA test method) have been used to determine the prevalence of murine viruses in NIEHS animals.

MAJOR FINDINGS AND PROPOSED COURSE: From October 1, 1981, to the present, a total of 2,013 serological tests were performed on 462 sera. Included were 153 rat sera (660 tests), 271 mouse sera (1,225 tests) and 38 hamster sera (128 tests). Supplier surveillance data indicates that: a) Incoming rats from Harlan are positive for Sendai, PVM, and RCV/SDA, b) Incoming rats from Charles River (Portage) are positive for KRV, and c) Incoming mice from Jackson are positive for PVM. Health surveillance data from NIEHS breeding colonies, and from experimental animals revealed that: a) Four different rat viruses (Sendai, PVM, KRV, and RCV/SDA), b) Three different hamster viruses (Sendai, PVM, and SV-5), and c) Four different mouse viruses (Sendai, PVM, MHV, and Reo-3) have been detected in animals housed in NIEHS facilities.

These data indicate that MHV is widespread in our mouse rooms and that some incoming mice had seroconverted to MHV positive within 3 weeks after arrival. These mice were housed under filter tops which should limit or reduce aerosol transmission. Considering our current husbandry techniques, these data suggest that MHV is extremely contagious, and that some route other than aerosol transmission is the major factor in the MHV epizootiology, or that the virus may be stable in the environment.

We will continue to screen our breeding colonies, incoming animals and experimental animals for the murine viruses. In addition, we will evaluate the factors which affect the distribution of infectious MHV among animals. Preliminary studies will be performed to demonstrate that we can clinically reproduce MHV. Normal and cortisone acetate stressed CD-1 mice, free of the murine viruses, will be experimentally infected with tissues from naturally infected mice. Clinically ill mice will be sacrificed and mice found dead will be necropsied and specimens collected and examined by histology and IFA to confirm the disease. IFA will permit studies on antigen localization during acute and persistent phases of the disease.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The quality of research generated at NIEHS is dependent upon the use of pathogen free animals. Viral infections in laboratory animals can profoundly alter biologic responses by altering the animals' metabolism, its immune system, and its response to neoplasia. MHV infection in normal adult mice is usually inapparent and may go undetected. MHV has been and continues to be a problem in our animal facilities by causing mortality in our nu/nu mice and by altering immunological studies in clinically normal mice. It is essential we know about the transmission and the stability of this virus in our environment if we are to eliminate these problems. CMB's ultimate goal is to eliminate all spontaneous diseases as sources of variability in our animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 22102-01 CMB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Characterization of a Coronavirus from Rabbits

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. D. Small Veterinary Medical Officer CMB NIEHS

OTHER:

COOPERATING UNITS (if any)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic & Research Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A coronavirus associated with rabbit cardiomyopathy has been previously described by the PI. Pleural effusion disease (PED) described by Danish workers is thought to be caused by the same agent. A comparative study of the 2 agents is beginning using techniques previously reported by the PI.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbits will be infected with serum supplied by the Danish workers and a pool of titered infectious serum will be developed. Cross neutralization studies will be done using hyperimmune serum previously collected or prepared in the present study. Immunofluorescence using techniques previously described will be used to localize antigen in the tissue. Other techniques as previously published by the PI may be used.

Previous Project No.: Z01 RS 00016-03 VR; 1978-79 DRS Annual Report.

MAJOR FINDINGS AND PROPOSED COURSE: Continuation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work should settle the issue of similarities or differences between rabbit coronavirus and the agent of PED.

HARLAN SPRAGUE DAWLEY, INC. - Indianapolis, Indiana 46229
(NIH-NO1-ES-8-2152)

TITLE: The Establishment of Specific Pathogen Free Rodent Stocks

CONTRACTOR'S PROJECT DIRECTOR: Peter D. James

PROJECT OFFICER (NIEHS): Conrad B. Richter, V.M.D., Chief
Comparative Medicine Branch

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $240,000

PROJECT DESCRIPTION

OBJECTIVES: Continuous need to expand the rodent gene pool at NIEHS for research in the IRP and the overall need for flexibility to meet all program needs requires that mechanisms exist to permit the acquisition of rodents from outside sources without endangering existing populations. To expedite this mission, the contract rederivation of new strains to assure their pathogen free status has been undertaken. Strains derived by this mechanism complement those that are handled inhouse. The process involves the hysterectomy of date mated pregnant females and the sterile grafting of pups from donor mothers to gnotobiotic foster mothers in isolators. Weanlings obtained by this process are inbred and the breeding nucleus expanded within the isolator to permit transfer of pedigreed stock to the Institute. Once these strains are established at the Institute, the isolator stock at the contractor's facility is phased out.

A second facet of the contract which accounts for approximately one-half of the total cost is the production of specific pathogen free F344/N rats for research programs in the IRP (LBNT, LP, LPFT). Current use rate from this part of the contract is approximately 12,000 rats per year.

MAJOR FINDINGS AND PROPOSED COURSE: During the current fiscal year to date three strains (LP/J, ST/bJ, and PL/J) have been rederived and received at the Institute. Four more strains are scheduled to be completed before the end of the fiscal year (T-stock, SM/J, RIIIs/J, and Elo). Three additional strains are rederived but may not be completed before the end of the fiscal year (B10.129/J, Nuc, Cc), and three additional strains are scheduled to replace the strains that have been completed. Considerable variation is experienced in the response of the different strains to this technology, and all variations cannot be simply explained on the basis of the inherent reproductive capacities of the different strains. For instance, LP/J, even though successfully rederived and transferred, is still of questionable status and requires close attention. Performance of the contractor is regarded as satisfactory and it is proposed to continue rederivation by this mode during the next fiscal year to the extent necessary to catch up on the backlog for new strain acquisitions; however, this part of contract work will be gradually phased out at least by the end of the next fiscal year.

The production of specific pathogen free F344/N rats has not been satisfactory since the contractor's production colony is infected with Sendai virus. The contractor is aware of this problem and has been advised that unless it can be

corrected by the end of the current fiscal year, this aspect of the contract will not be renewed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is essential that the Institute research enjoy the flexibility to proceed with new projects in science as rapidly as possible. The ability to acquire and manipulate "new" mammalian genes is a significant part of Institute research programs. At the same time it is recognized that uncontrolled introduction of new strains of rodents constitutes a major threat to existing populations by way of adventitious epidemic pathogens. The present contract presents one aspect of a multi-faceted program operated or proposed by CMB to achieve these goals. At the same time these procedures complement CMB's targeted elimination of rodent pathogens from experimental stock at the Institute. The destructive influence of these diseases on Institute research programs has been well demonstrated.

TOXICOLOGY RESEARCH AND TESTING PROGRAM

TOXICOLOGY RESEARCH AND TESTING PROGRAM

Summary Statement

The Toxicology Research and Testing Program, an integral component of the National Toxicology Program, develops scientific information about potentially toxic and hazardous chemicals which can be used for protecting the health of the American people and for the primary prevention of chemically-induced disease. TRTP concentrates activities in toxicology research, testing and test development/validation efforts, and provides toxicological information needed by research and regulatory agencies. Four specific aims have been identified:

-- To expand the toxicological profiles of the chemicals nominated, selected, and being tested.

-- To increase, as necessary and as funds permit, the number and rate of chemicals under test.

-- To develop and validate a series of tests/protocols more appropriate for regulatory needs.

-- To establish and use a coordinated communications network to collect, evaluate, and disseminate toxicological information.

To accomplish these major goals, the program segments are grouped into two categories -- toxicologic research and testing, and coordinative management activities.

The research branches embrace Cellular and Genetic Toxicology (Dr. R. Tennant), Chemical Pathology (Dr. E.E. McConnell), Systemic Toxicology (Dr. J.A. Moore), and Carcinogenesis and Toxicology Evaluation (Dr. J.A. Moore, Acting). The management branches involve Program Operations (Dr. J.F. Douglas), Program Resources (Dr. C. Grieshaber), and Data Management and Analysis (Dr. D. Hoel, this branch is part of the Biometry and Risk Assessment Program). [Each of these discipline areas and their accomplishments are described separately in the sections that follow this overview.]

Individual NTP scientists have been appointed as leaders of major program segments. Each scientist serves as the center for a particular program activity and is responsible for developing (in collaboration with other NTP colleagues) the subprogram objectives and the implementation plan, as well as the coordination and supervision of the program work. Further, the program leaders are responsible for the development and supervision of contracts that extend these activities or that perform in-depth toxicologic characterization of chemicals.

The strategy for test development and validation examines existing and emerging methodologies to identify those which may be adequately sensitive and reproducible. Those offering improvement over older methods will be selected for validation. When basic research findings suggest new areas of toxicology testing, TRTP will undertake the appropriate methods development and validation. Existing methodologies that are being examined for modification include techniques used to detect impaired liver or kidney function and neurobehavioral

toxicity; and new areas for methods development and validation include behavioral teratology, short-term tests for presumptive carcinogenic potential, and cardiovascular toxicology.

A logical mutagenicity five system testing battery has been implemented for all chemicals selected into the toxicology and carcinogenesis bioassay program; likewise, chemical disposition profiles are accomplished for those chemicals going into the chronic testing phase. Fertility and reproductive assessment are done on all chemicals in the prechronic (90-day) studies. In the area of immunological toxicology a testing panel has been established to determine effects on the rodent immune system and to further decipher the possible link between immunologic effects and the carcinogenic response.

To advance the current testing procedures for detecting chemically-induced cancer in rodents, the TRTP has begun to initiate and integrate certain innovations into the long-term (2-year) carcinogenesis bioassay.

Biomathematical simulations aimed at improving the basic experimental design of the two-year bioassay provides information useful for low-dose extrapolation while retaining the power of the bioassay for detecting carcinogenic effects. Three and four-dose designs were examined. The optimal design involved four groups (control plus low, medium, and high dose). The maximum tolerated dose (MTD) would be the high dose, the middle dose one-half the MTD, and the low dose 10-30% of the MTD. The most appropriate designs are being considered for implementation in the near future for long-term carcinogenicity bioassays.

The traditional pathology procedures in two-year rodent bioassays requires that 42 sections from 32 tissues be examined microscopically from all animals that die during the bioassay and from those at the end of the 104 week treatment period. Analyses of the results from previous NCI and NTP bioassays indicate that significant reductions in the number of tissues examined could be implemented without compromising the ability to detect chemically-induced tumors. Further, the quality of the toxicologic pathology will be markedly improved through examination of some animals at a time period earlier than 24 months, when normal aging lesions often interfere with the detection and interpretation of chemical related lesions.

Short term in vivo rodent liver carcinogenesis models are being refined to help clarify the nature of carcinogenic responses associated with two year rodent bioassays. A major objective will be to assess the ability of selected chemicals to act as initiators, promoters, or complete carcinogens in these models. Initial emphasis will be directed toward further model development including assessment of chemical dosimetry. The research will attempt to quantitatively assess response through the use of preneoplastic markers and correlate the results with histomorphologic tumor endpoints. Initial selection of chemicals will focus on those known to induce liver tumors in rodents, taking into account their genetic toxicity.

To undergird these activities, research projects are being pursued to better optimize the available resources. Particular chemical class studies have been designed to better define structure activity correlations; these for instance involve projects on benzidine and benzidine-based dyes, on phthalic acid esters, and on halogenated hydrocarbons. The manifold chemicals in these classes are produced in large volumes, have multiple uses, and considerable segments of the

occupational work force and the general population receive exposure to these end products. Establishing basic toxicology principals reduces the necessity to rotely test all potentially hazardous chemicals within each of these structural classes. These research and test development advances reflect shortly thereafter in the testing activities per se.

This portion of the NIEHS, that is the TRTP, represents in essence the NIH component of the National Toxicology Program, contributing approximately 87% of the total NTP resources.

DEPARTMENT OF ENERGY - OAK RIDGE NATIONAL LABORATORY
(222 Y01-ES-1-0072)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: John Wassom

PROJECT OFFICER (NIEHS): J.E. Huff, Ph.D.

DATE INTERAGENCY INITIATED: FY 1971

CURRENT ANNUAL LEVEL: $360,000

PROJECT DESCRIPTION

Chemical Mutagenesis Literature -- Supported by the NTP, the Environmental Mutagen Information Center (EMIC) collects, organizes, and disseminates primarily published information on chemicals tested for mutagenicity. Located since inception in 1969 at the Oak Ridge National Laboratory, the EMIC computerized data file contains 40,841 (May 1982) records, most of which are available online from TOXLINE (at the National Library of Medicine) and from RECON (at the ORNL). Each record contains bibliographic information, assay systems, and keywords defining agents tested and organisms studied. All articles are obtained prior to entry onto computer and are on file at the EMIC. The number of unique chemicals identified from these 40,841 documents equals 13,325.

DEPARTMENT OF ENERGY - OAK RIDGE NATIONAL LABORATORY
(222 Y01-ES-1-0073)

TITLE: Environmental Teratology Information Center

CONTRACTOR'S PROJECT DIRECTOR: John Wassom

PROJECT OFFICER (NIEHS): J.E. Huff, Ph.D.

DATE INTERAGENCY INITIATED: FY 1975

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

Chemical Teratogenesis Literature -- Developed and supported by the NTP since 1975, the Environmental Teratology Information Center collects, organizes, and disseminates information on chemicals tested for teratogenicity. The ETIC data file contains 25,799 records, the majority of which are available online from TOXLINE and from RECON. The number of unique chemicals identified from these 25,799 documents equals 5,407 (as of May 1982).

ETIC, located at NIEHS, has established a microform document library containing copies of 20,615/25,799 (80%) papers referenced in the computer file. Each citation has been indexed with all bibliographic information, common and taxonomic name of test object, and Chemical Abstract Service (CAS) Registry Number. Titles and abstracts may be searched using key words. Chemicals in the ETIC Agent Registry may be searched by primary name, synonym, CAS number, fragment as a part of a chemical name, and molecular formula.

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH

SUMMARY STATEMENT

The Carcinogenesis and Toxicology Evaluation Branch (CTEB) (1) conducts applied research intended to develop and validate improved toxicity testing methodologies, establish short-term and screening test systems, and improve interpretation of long-term bioassay results; (2) collaborates as toxicology experts with other scientific staff in the National Toxicology Program involved in test development and validation and test protocol preparation; and (3) monitors testing programs to assure the quality and validity of the toxicity studies.

This is the second year in existence of this branch and the major efforts during this year were the evaluation of toxicologic and carcinogenic effects of chemicals conducted through contracts with various laboratories. The in-house research activities were initiated by a number of scientists in the branch and most of these activities are collaborative efforts within NTP disciplines and, to some extent, with NIEHS intramural scientists.

Extramural Research: All extramural research activities were undertaken through contract mechanisms. The following are the highlights of various activities under this category:

- Toxicity and carcinogenicity testing of 136 chemicals is being studied under NTP Basic Ordering Agreement and Tracor Jitco contracts. These studies are at prechronic and chronic phases. The branch has developed protocols for a number of chemicals for which the contracts are planned to be awarded this fiscal year. A number of technical reports from TJ contracts were prepared for studies which have been completed.

- The biologic, pharmacologic, and toxicologic properties of 8-methoxypsoralen with and without ultraviolet A light are being studied in the Fischer 344 rat and the HRA/skh hairless mouse. These studies are being conducted through five different contracts. A conference of the toxic properties of psoralens was held at the NIEHS in 1982 (one support contract) and the proceedings of this conference will be published in the Journal of the National Cancer Institute (Dr. Dunnick).

- Toxicity and carcinogenic evaluation of benzidine congener dyes - The objective of this program is to develop an integrated body of scientific knowledge concerning the absorption, metabolism and excretion; the genetic toxicology and the in vivo carcinogenicity of the benzidine congeners and selected prototypical dyes. Through the judicious selection of chemicals for testing it will be possible to establish basic principles which can be applied to the entire class of benzidine-based dyes. This is a collaborative effort among various disciplines within the NTP and through contract mechanisms. Significant progress was made in the areas of chemical disposition and metabolism and in vivo toxicity and carcinogenicity testing through both intramural and contract research programs. Contracts for genetic toxicology testing have been awarded and research is now underway (Dr. Mennear).

- The effect of microencapsulation on the chemical stability and bioavailability of 2,6-xylidene is being evaluated in collaboration with Drs. Jameson and Matthews (Dr. Melnick).

- Studies are underway to determine if there is increased sensitivity of laboratory animals to potential carcinogenic and toxicologic effects of selected chemicals exposed at various levels during their in utero development plus post-natal life of two years as compared to the animals exposed only during post-weaning time of two years. This study is performed through a contract with Battelle Columbus Laboratories. Phenytoin, ethylenethiourea and Firemaster FF-1 (PBB's) are being studied under this contract. At present, these studies are at prechronic and chronic phases (Dr. Chhabra).

Intramural Research: The following research projects are being conducted in-house by the branch scientists:

- Current and novel clinical chemistry assays to evaluate responsiveness to toxic chemicals, reproducibility, sensitivity, prognostic value for target organ toxicity, and practicality for contract use will continue to be studied in rodents. Optimum conditions for cholinesterase, sorbitol dehydrogenase and gammaglutamyl-transpeptidase assays in rodent blood are being documented (Dr. Dieter).

- Effects of hexane on the neurotoxicity and disposition of various organophosphorous pesticides are being studied at Duke University in collaboration with Dr. Abou-Donia (Dr. Abdo).

- Studies on the toxic effects of phthalate esters on energy coupling in rat liver mitochondria are being completed in collaboration with Dr. Schiller (Dr. Melnick).

- The effects of neurotoxic agents on the in vitro activity of microtubules are being studied at NIEHS in collaboration with Dr. Chignell (Dr. Irwin).

- The in-house research efforts are being directed to assess the sensitivities and versatilities of various tests for detecting subtle kidney injury; to understand mechanisms of chemical nephropathy elicited by nephrotoxic chemicals, and also the acute and subchronic toxic effects of the pesticide 1,2-dibromo-3-chloropropane (DBCP) and structurally-related compounds are studied from functional and mechanistic viewpoints. The biological and toxic effects of di(2-ethylhexyl)phthalate and butyl benzyl phthalate are being studied to determine dose-response relationships and mechanisms of deleterious effects (Dr. Kluwe).

- In vivo and in vitro studies with mercuric chloride were completed to investigate the mechanism(s) of immunotoxicity. Mercury inhibited cell-mediated immunity at concentrations below effects on humoral-mediated immunity, inhibited glycolysis in T-cells, and acted by destroying sulfhydryl groups necessary for cellular metabolism and immune responsiveness (Dr. Dieter).

- Studies with male rats demonstrated decreases with age in intermediary glucose metabolism of pulmonary macrophages and thymus, consistent with a loss of cell-mediated immune function in aged rodents and humans (Dr. Dieter).

- Elucidation of routine clinical sampling of rats to establish optimum procedures for obtaining accurate assessment of blood parameters in toxicity testing in collaboration with Dr. R. Maronpot (Dr. Eastin).

- The comparative subchronic toxicities of the benzidine dye, C.I. Direct Blue 6, and benzidine were studied in rats to determine if the carcinogenicity reported for the dye is attributable to factors other than biotransformation to the carcinogenic aromatic amine. (Dr. Mennear).

- Studies are underway to assess the effects of dimethyl methyl phosphonate (DMMP) on the reproductive system of the male rat and these studies include: histopathologic examination of the reproductive organs; evaluation of sperm count and morphology; and a mating trial with examination of the pups for evidence of abnormalities (Dr. Dunnick).

Other Activities:

Dr. Kluwe: Developed future research and testing plans on phthalates for NTP. Participated in a World Health Organization-sponsored independent analysis of the scientific evidence pertaining to the carcinogenic potentials of several chemicals (International Agency for Research on Cancer, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, October, 1981, Lyon, France).

Dr. Dunnick: Serving on the Ad Hoc Interagency Dermatology Working Group to coordinate dermatology research at the NIH and throughout the government.

Dr. Dieter: Serving as Project Officer for the Toxicology Data Management System (TDMS) and the implementation of TDMS by the National Toxicology Program.

Drs. Abdo, Chhabra and Dunnick: Were certified in General Toxicology by the American Board of Toxicology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21001-02 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of chemical nephrotoxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.: William M. Kluwe Pharmacologist, TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
TRTP/NIEHS

SECTION
Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/8	1/8	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Time- and dose-dependent effects of selected nephrotoxic agents on the ultrastructure and biochemical status of target and non-target cells in the kidney are evaluated to determine basic mechanisms of injury to various renal cell populations. Comparisons are made between chemical structures and the types of subcellular lesions induced, or the target cells affected, to elucidate common pathophysiological sequences of chemically-induced renal cell injury.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: At several times post-dosing and at multiple dose levels (range of non-toxic to maximally-toxic), evaluations are made of organ morphologies by light microscopy and of subcellular organization by transmission electron microscopy. Evaluations are also made at the same times of biochemical and physiological parameters indicative of the status of cell function in general and subcellular organelle (e.g., plasma membrane, mitochondria) lability more specifically.

MAJOR FINDINGS AND PROPOSED COURSE: Many nephrotoxic organohalide compounds that selectively injure cells of the pars recta (S_3) initially cause vesiculation of the cytoplasm in the apical portion of the cell. Later-appearing morphological effects include microbody proliferation, mitochondrial swelling, increased smooth endoplasmic reticulum and aggregation of chromatin at the periphery of the nucleus.

Assessments are being made of ATP concentration, mitochondrial function, pinocytotic reabsorption, lysosomal lability, endoplasmic reticulum integrity and enzymatic activities and incorporation of precursors into RNA, DNA, protein and lipid to correlate the morphological changes with biochemical effects and to suggest mechanisms of action.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Kidney disease is a major cause of debilitation in the U.S. Though the extent of chemicals as causative agents in toxic nephropathy in humans is unknown, animal studies suggest considerable susceptibility of mammalian kidneys to halogenated hydrocarbons and organic amines. Mechanistic studies are necessary to assess experimental animals as models of human response to nephrotoxicants and for the extrapolation of animal safety studies to the human situation.

PUBLICATIONS

Kluwe, W. M. and Hook, J. B.: Effects of Environmental Chemicals on Kidney Metabolism and Function. Kidney Intl. 18: 648-655, 1980.

Kluwe, W. M. and Hook, J. B.: Metabolic Activation of Nephrotoxic Haloalkanes. Federation Proc. 39: 3129-3133, 1980.

Kluwe, W. M. and Hook, J. B.: Potentiation of acute Chloroform Nephrotoxicity by the Glutathione Depletor Diethyl Maleate and Protection by the Microsomal Enzyme Inhibitor Piperonyl Butoxide. Toxicol. Appl. Pharmacol. 59: 457-466, 1981.

Kluwe, W. M.: The Nephrotoxicities of Low Molecular Weight Halogenated Aliphatic Solvents, Pesticides and Chemical Intermediates. In Toxicology of the Kidney, (J. B. Hook and R. L. Dixon, eds.), Raven Press, New York, pp. 179-226, 1981.

ADDITIONAL PROJECTS

1. Chemical Manager for the following chemicals:

Agent	Current Testing Phase
Chlorobenzene	Chronic
Benzaldehyde	Chronic
Diallylphthalate	Chronic
Nitrofurantoin	Chronic
Nitrofurazone	Chronic
Bromobenzene	Prechronic
Diethylphthalate	Prechronic
Methylphenidate	Pretesting

2. Phthalate Ester Toxicology

An evaluation was made of the adequacy of available toxicology information on ortho-phthalate esters. A NTP-sponsored conference on phthalate esters was held and plans for future NTP endeavors in phthalate ester research were formulated.

PUBLICATIONS

Kluwe, W. M.: An Overview of Phthalate Ester Pharmacokinetics in Mammalian Species. Environ. Health Persp. (in press), 1982.

Kluwe, W. M., McConnell, E. E., Huff, J. E., Haseman, J. K., Douglas, J. F. and Hartwell, W. V.: Carcinogenicity Testing of Phthalate Esters and Related Compounds by the National Toxicology Program. Environ. Health Persp. (in press), 1982.

Author: Kluwe, W. M. Phthalic Acid Esters: Part I, Toxicological Evaluation, (NTP-81-49), U.S. Department of Health and Human Services, PHS, NIH, National Toxicology Program, Research Triangle Park, NC, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21011-02 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Comparative Toxicities and Carcinogenicities of C.I. Direct Blue 6 and Benzidine in Rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John H. Mennear	Expert	TRTP	NIEHS
Co-PI:	Bhola N. Gupta	Pathologist	TRTP	NIEHS
Other:	Martha Harris	Bio. Lab. Tech.	TRTP	NIEHS

COOPERATING UNITS (if any)

Biometry

LAB/BRANCH
TRTP/NIEHS

SECTION
Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75 (technician)

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

C.I. Direct Blue 6, a commercially available textile dye, has been reported to produce hepatocellular carcinoma in rats fed diets containing from 1,500 to 3,000 ppm of test chemical. The dye is derived from benzidine, a known hepatocarcinogen in rats, and when administered orally to rats the dye is metabolized to the carcinogen. Hepatocellular carcinomas induced by the dye appeared after only five weeks of treatment, an onset time which is much shorter than has ever been reported for benzidine-induced carcinomas in rats. This observation raises the question of whether the dye-induced carcinomas were mediated through the dye per se, benzidine produced through the biotransformation of the dye, or some other metabolite. When considered on a molar basis, the benzidine equivalents of the dye were far in excess of any dosage levels of benzidine reported in the literature. The objective of this study was to compare the toxicities and carcinogenicities of benzidine and Direct Blue 6 (in molar equivalent doses with respect to benzidine). The results show that the dye is not more toxic than a molar equivalent dose of benzidine. Further, neither benzidine nor direct Blue 6 produced hepatocellular carcinoma during this 13-week study.

PROJECT DESCRIPTION

METHODS EMPLOYED: Benzidine and C.I. Direct Blue 6 were compared with respect to effects on: 1) food consumption, 2) survival, 3) organ function, 4) urinary excretion of benzidine metabolites, and 5) the development of neoplastic and preneoplastic lesions.

MAJOR FINDINGS AND PROPOSED COURSE: C.I. Direct Blue 6 (70 to 186 mg/kg/day administered in drinking water) was compared to roughly molar equivalent doses of benzidine (10 to 31 mg/kg/day). Dose-related decreases in survival, food consumption and body weight gains were produced by both chemicals, however, benzidine had a far greater effect on these parameters than did Direct Blue 6. Similarly, the effect of benzidine on serum concentration of gamma glutamyl transpeptidase was greater than that of Direct Blue 6. Neither chemical produced hepatocellular carcinoma, however, both caused the production of regenerative nodules. This liver lesion is indicative of tissue regeneration in response to hepatic toxicosis. The magnitude of these lesions was dose related and more severe in benzidine treated animals. Both chemicals were found to have produced hyperplasia and adenomas of the zymbal gland. These changes appeared only in rats that survived to the end of the study and the incidences were equivalent between the two chemicals. The project is completed and a publication is now in preparation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is the intramural portion of the National Toxicology Program's benzidine congener dye initiative. The results will be used to facilitate the interpretation of the pharmacokinetics and genetic toxicology portions of the initiative. The results will also aid in the interpretation of the earlier Direct Blue 6 study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21021-01 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Changes in Intermediary Metabolism in Lymphoid Organs of Aging F344/N Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael P. Dieter	Physiologist	CTEB	NIEHS
Other:	Ralph Wilson	Technician	CPB	NIEHS
	Linda Birnbaum	Pharmacologist	STB	NIEHS

COOPERATING UNITS (if any)

Systemic Toxicology Branch, National Toxicology Program, NIEHS

LAB/BRANCH
Carcinogenesis and Toxicological Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Key enzymes from the hexose monophosphate shunt, glycolysis and the tricarboxylic acid cycle were compared in lymphoid components of 18 and 24 month old rats. Metabolic alterations that result in critical immunobiochemical lesions may be responsible for the decline in host resistance and increase in autoimmunity common to the aging process. Enzyme analysis in thymus and pulmonary macrophages indicated depression of biosynthetic pathways suggestive of decline of cell-mediated function with age, whereas splenic enzyme activity did not decline with age. The latter interpretation was confounded by the heterogeneity of the spleen cell population but is consistent with unchanged humoral immune function during aging.

PHS-6040
(Rev. 2-81)

METHODS EMPLOYED : Current biochemical methods utilizing conventional UV and centrifugal analysis spectrometry, and radioenzymatic assays are utilized.

MAJOR FINDINGS AND PROPOSED COURSE: Glucose metabolizing enzymes in thymus and spleen were conversely affected by aging. Between 18 and 24 months, key glycolytic enzymes in rat thymus (pyruvate kinase and lactate dehydrogenase) decreased 50%, whereas enzymes from all three pathways increased 50-100% in spleen. In addition, significant changes with age occurred in all three enzymatic pathways of glucose metabolism in pulmonary macrophages as shown by 61%, 58%, and 33% decreases in glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, and malic dehydrogenase, respectively.

Further biochemical analyses of T-cells, B-cells, and macrophages in aging rats will be performed as well as immune functional assays to determine the nature of the relationship between these variables.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information on biochemical alterations of lymphoid organs during aging may be useful for therapeutic intervention and will improve our understanding of the decline in host resistance that occurs during aging.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21022-01 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Effects of Immunotoxic Chemicals on Intermediary Metabolism of Mouse Lymphoid Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael P. Dieter	Physiologist	CTEB	NIEHS
Other:	Ralph Wilson	Technician	CPB	NIEHS

COOPERATING UNITS (if any)
Systemic Toxicology Branch, National Toxicology Program, NIEHS

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The correlation between chemically-induced immune defects and biochemical defects in lymphoid tissues (thymus, spleen, bone marrow) and specific-populations of lymphoid cells (pleuripotent stem cells, granulocyte-macrophage precursors, T-cells, B-cells, peritoneal and pulmonary, macrophages) were investigated in in vitro and in vivo studies. Rate limiting enzymes in the hexose monophosphate shunt, glycolysis and the tricarboxylic acid cycle, and marker enzymes in macrophages, have been assayed. Change in substrate flow through these biosynthetic pathways were caused by immunotoxic chemicals (mercuric chloride, methyl and ethyl carbamate, DES, asbestos) and tissue-specific pathway inhibition was correlated with functional defects in the immune system.

PHS-6040
(Rev. 2-81)

METHODS EMPLOYED : Current biochemical methods utilizing conventional UV and centrifugal analysis spectrometry, and radioenzymatic assays are utilized.

MAJOR FINDINGS AND PROPOSED COURSE: Assay of six rate limiting enzymes for glucose metabolism via monophosphate shunt, glycolysis, or tricarboxylic acid cycle were standardized in bone marrow, macrophages, thymus, and spleen.

Lymphoid tissues from mice treated with various classes of chemicals revealed patterns of response consistent with specific T-cell immunotoxicity. Mercuric chloride, methyl, and ethyl carbamate, diethlystilbestrol, alpha-dinestrol, and 17 β-estradiol caused defects in T-cell immunity and specific inhibition of enzymes from the glycolytic pathway in thymus. Lymph nodes are serving as model tissues to represent B-cell responses to chemical insult. In vitro cell cultures of thymus, spleen, or lymph nodes are being utilized to investigate the relationships between intermediary metabolism and T-cell or B-cell mitogenic responses.

I will continue to participate in a screening program for immunotoxic chemicals by providing correlative evidence of metabolic derangement with immune dysfunction in specific populations of lymphoid cells and macrophages. Whenever feasible and promising, mechanistic studies to elucidate the metabolic basis of specific immunotoxicity will be pursued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE : Information regarding the immunotoxicity of each chemical may be useful in therapeutic intervention and will provide another sensitive measure of the potential chemical hazard to man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21023-01 CTEB

PERIOD COVERED
March 30, 1982 to July 30, 1982

TITLE OF PROJECT (80 characters or less)

A study of the effects of dimethyl methyl phosphonate on the reproductive system of the Fischer 344 male rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	June K. Dunnick	Chemist	TRTP	NIEHS
Co-PI:	Bhola N. Gupta	Pathologist	TRTP	NIEHS
Co-PI:	James C. Lamb, IV	Research Biologist	TRTP	NIEHS
Other:	Martha Harris	Technical Supervisor	TRTP	NIEHS
	John A. Moore	Chief, Systemic Tox.	TRTP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dimethyl methyl phosphonate (DMMP) is a nerve gas simulant used in the U.S. Armed Forces. Mild testicular lesions were diagnosed in the Fischer 344 male rat after the 90-day subchronic study. This study is designed to further assess the effects of DMMP on the reproductive system of the male rat. DMMP will be administered by gavage for 90 days at 0, 250, 500, 1,000 and 2,000 mg/kg. On day 84 the rats will be mated. The female rats will be sacrificed 14 days after mating and pups examined. Male reproductive organs will be examined for sperm morphology and counts; histopathology will be performed on the male reproductive organs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: This study is designed to determine the effects of dimethyl methyl phosphonate (DMMP) on the reproductive system of the male Fischer 344 rat.

METHODS EMPLOYED: Administration of DMMP by gavage for 13 weeks; mating trial; determination of sperm morphology and epididymal sperm count; gross and histopathology on male reproductive organs; hormone assays.

MAJOR FINDINGS AND PROPOSED COURSE: The study is still in the dosing phase, and effects on the male reproductive system will be measured in the next several months.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dimethyl methyl phosphonate (DMMP) is a nerve gas simulant used in the U.S. Armed Forces. Current military uses of DMMP include: a simulant for anticholinesterases for testing vapor samplers; and a simulant for suitability of filters and filter canisters for military protective masks. Little information is available on the toxicologic properties of DMMP, and the U.S. Armed Forces has asked the National Toxicology Program to test this compound. This study is designed to test the effects of DMMP on the reproductive system, and will serve as one part of the NTP's overall assessment of DMMP. Other studies underway include a two year bioassay in the Fischer 344 rat and $B6C3F_1$ mouse.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30100-03 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Toxic effects of 1,2-dibromo-3-chloropropane on the urogenital system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	William M. Kluwe	Pharmacologist, TRTP	NIEHS
Others:	James C. Lamb, IV	Biologist, TRTP	NIEHS
	Bhola N. Gupta	Pathologist, TRTP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
TRTP, NIEHS

SECTION
Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-1/8	1/8	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The acute and subchronic toxic effects of the pesticide 1,2-dibromo-3-chloropropane (DBCP) and structurally-related compounds are studied from functional and mechanistic viewpoints. A reported chemo-sterilant in humans, DBCP is no longer manufactured in the U.S., but its presence in ground water and on edible imports and its illegal bulk transport into certain areas of the U.S. require its further toxicological characterization. Effects of DBCP on hepatic, renal and reproductive functions are evaluated at several dose levels, after various treatment regimens and under differing conditions such as age, chemical or physical stress and the like. The distribution and disposition of DBCP is being studied in rats, as well as selected aspects of its metabolism and the effects of metabolic modulation on DBCP toxicities.

Comparative toxicities of DBCP and its metabolites are being evaluated to ascertain the toxic chemical moiety and to predict whether structurally similar chemicals would produce the same toxic effects as DBCP.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Toxic effects are being studied in male and female Fischer 344 rats using a variety of functional, biochemical and pathological techniques. Disposition, distribution and metabolism studies are conducted in, or with tissues from, male Fischer 344 rats by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Acute intoxication with DBCP causes dose-dependent injury to the kidney, testis, epididymis and liver. Effects on the liver, epididymis and kidney appear to be reversible, but testicular damage is progressive and may be irreversible following significant acute injury. The acute toxic manifestations of DBCP treatment bear many similarities with the acute toxic effects of the DBCP metabolites epi- and alpha-chlorohydrin and ß-chlorolactic acid, but not with oxalic acid, another DBCP metabolite. These results suggest that DBCP, epichlorohydrin and alpha-chlorohydrin may exert their effects via a common pathophysiological mechanism. DBCP nephrotoxicity and testicular toxicity is blunted by pretreatment with the microsomal enzyme inducer phenobarbital, but enhanced by pretreatment with cobaltous chloride or by partial hepatectomy. DBCP metabolism, therefore, appears to be involved in the expression of toxicity, though the mechanism of metabolic modulation remains to be elucidated. DBCP is detoxified by conjugation with hepatic glutathione, and the threshold acute toxic dose of DBCP coincides with the dose that significantly depletes hepatic glutathione. Immature rats (24 days old) are relatively resistant to the acute toxic effects of DBCP. Repeated exposure to acutely less-than-toxic DBCP doses produces a transient period of infertility in male rats, but no change in epididymal sperm number, motility or morphology. Future studies will continue to characterize the dose-response relationship for DBCP and examine cumulative toxic effects. Pharmacokinetic studies will determine tissue repositories, the relationships of metabolite patterns to tissue injuries and the propensity of DBCP or metabolites to interact with genetic materials. The basis for resistance of young animals to acute DBCP toxicity will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Characterization of the toxic effects of DBCP and elucidation of the mechanisms of action of this and similar toxic halocarbon compounds will allow better estimates of human risk to be made. Observance of reduced fertility at doses below those which reduce sperm number suggest that sperm counts (currently used as an index of human DBCP toxicity) may be inadequate to ensure safe human exposures. Similarities between the toxic actions of DBCP, epi- and alpha-chlorohydrin indicate the possibilities of "DBCP-like" effects for other chemicals that have similar structures.

PUBLICATIONS

Kluwe, W. M.: Acute Toxicities of 1,2-Dibromo-3-chloropropane in the Fischer 344 Male Rat. I. Dose-Response Relationships and Differences in Routes of Exposure. Toxicol. Appl. Pharmacol. 59: 71-83, 1981.

Kluwe, W. M.: Acute Toxicities of 1,2-Dibromo-3-chloropropane in the Fischer 344 Male Rat. II. Development and Repair of the Renal, Epididymal, Testicular and Hepatic Lesions. Toxicol. Appl. Pharmacol. 59: 84-95, 1981.

Kluwe, W. M., Greenwell, A. and Harrington, F. W.: Relationship of tissue non-protein sulfhydryls to the acute toxic effects of 1,2-dibromo-3-chloropropane. J. Pharmacol. Exp. Ther. 220: 399-405, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30101-03 CTLB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Renal function tests as indicators of nephrotoxicity.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William M. Kluwe Pharmacologist, TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
TRTP/NTP

SECTION
Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/8	1/8	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Renal function tests are conducted after single or multiple exposures of rats to a variety of chemical agents to assess the sensitivities and versatilities of the various tests for detecting subtle kidney injury. When appropriate, new or improved methodologies are designed and evaluated. The development of resistance to injury upon repeated chemical exposure and the effect of such or other chemical stresses to the body are also studied.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Following chemical exposure, the animals are placed in metabolic cages and urine is collected under appropriate conditions. Urinalyses of varying completeness are performed to assess the functional status of the kidney. Animals are sacrificed as necessary and the morphological appearance of the kidney compared to the functional effects of chemical treatment.

MAJOR FINDINGS AND PROPOSED COURSE: Tests that measure functional capacities of the kidney are the most sensitive and versatile indicators of subtle kidney injury, while standard tests such as blood urea nitrogen and serum sodium concentrations are relatively poor diagnostic tools.

The resistance to Mercuric chloride ($HgCl_2$) nephrotoxicity induced by repeated treatment with $HgCl_2$ does not extend to other chemical nephrotoxicants that damage the same or dissimilar sections of the proximal tubule as does $HgCl_2$.

Since many nephrotoxicants appear to interfere with protein handling by the proximal tubules, comparisons are being made by electrophoretic methods of the distribution of low molecular weight proteins from the urines of rats treated with selected nephrotoxicants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Sensitive and appropriate endpoints, as are being studied or developed in this project, are essential to the conduct of meaningful toxicology studies, including those conducted by the National Toxicology Program. In addition, sensitive function tests may suggest mechanisms of nephrotoxic action.

PUBLICATIONS

Kluwe, W. M., Renal Function Tests as Indicators of Kidney Injury in Subacute Toxicity Studies. Toxicol. Appl. Pharmacol. 57: 414-424, 1981.

Kluwe, W. M.: Rapid, Automated Measurements of Urinary Protein and Glucose Concentrations. Pharmacol. Meth. 5: 395-400, 1981.

Kluwe, W. M.: The development of resistance to nephrotoxic insult: changes in urine composition and kidney morphology upon repeated exposures to mercuric chloride or biphenyl. J. Toxicol. Environ. Health, (in press), 1982.

Kluwe, W. M.: Developed resistance to mercuric chloride: failure to protect against other nephrotoxicants. Toxicol. Lett. (in press), 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30102-03 CTEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interactions between halogenated aliphatic chemicals and renal tubular cells in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: William M. Kluwe Pharmacologist, TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
TRTP/NIEHS

SECTION
Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/8	1/8	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemical and physiological functions of renal cells are studied concurrent with, or following, in vitro exposure to nephrotoxic chemicals. The elicited effects are correlated with morphological alterations to assess subcellular mechanisms of action. Parallel studies are conducted in intact animals (in vivo) to assure the relevancy of the effects studied in vitro and to determine the role of extrarenal factors in the development of chemical nephropathy.

The in vitro environment (e.g., pH, electrolytes, cofactors, energy substrates) is manipulated to suggest biochemical mechanisms of action.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparations of renal tissue (e.g., slices, isolated cells or tubules) are combined with nephrotoxic chemicals *in vitro* and several functional (e.g., electrolyte transport, energy metabolism) and biochemical parameters are monitored. The major source of renal tissue is adult, male, F344 rats.

MAJOR FINDINGS AND PROPOSED COURSE: Many nephrotoxic halogenated aliphatic chemicals cause rapid, concentration-dependent depressions of renal proximal tubular cell function *in vitro*. The correlation between functional disturbances produced *in vitro* and *in vivo* is good, though the effects are demonstrable much more rapidly *in vitro* than *in vivo*.

Future experiments will more closely evaluate the morphological effects produced *in vivo* and *in vitro* at early time periods post-exposure and will ascertain the earliest functional abnormalities produced *in vivo*. The metabolism and degradation of toxic organohalides by kidney cells *in vitro* will also be studied and the use of non-rodent (e.g., humans or other primates) tissues will be explored.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Many nephrotoxic halogenated aliphatic chemicals are commonly used as industrial intermediates or as pesticides, resulting in widespread environmental distribution and human exposure. By studying the interactions of these agents with the target cells (kidney proximal tubular epithelium) *in vitro*, mechanisms of action can be suggested, eventually leading to a better estimation of their potential for human injury.

PUBLICATIONS

Kluwe, W. M.: Mechanisms of Acute Nephrotoxicity: Halogenated Aliphatic Hydrocarbons. In Drugs and Environmental Toxicants, (G. A. Porter, ed.), Elsevier-North Holland, 1982 (in press).

Kluwe, W. M., Harrington, F. W. and Cooper, S. E.: Toxic effects of organohalide compounds on renal tubular cells *in vivo* and *in vitro*. J. Pharmacol. Exp. Ther. (in press), 1982.

INTRAAGENCY AGREEMENT
222Y01-ES-20081

TITLE: Toxicology Data Management System

PROJECT OFFICER (NCI/NTP): Michael P. Dieter (NIEHS)
Albert J. Konvicka (NCTR)

DATE CONTRACT INITIATED: January 15, 1982

CURRENT ANNUAL LEVEL: $1,386,000

PROJECT DESCRIPTION

OBJECTIVES: NCTR will implement and maintain automated support of the information processing requirements (Toxicology Data Management System [TDMS]) for the animal bioassay portion of the NIEHS Toxicology Research and Testing Program, a major operating component of the National Toxicology Program. TDMS will replace the Carcinogenesis Bioassay Data System and then will serve as the principal data base for all animal bioassays. To accomplish TDMS implementation, it is necessary to purchase the appropriate hardware components and prepare and validate the appropriate computer programs for data collection and data retrieval. Data retrieval capability must be continually available for transmittal, examination, and utilization by NIEHS, NCTR, and participating contract laboratories.

METHODS EMPLOYED: Generally, for each contract laboratory, specific requirements for implementation of the TDMS will be done in three phases: 1) introduction of manual data collection forms; 2) installation of available microprocessor terminals and software; and 3) complete automated support of all NIEHS bioassay studies.

MAJOR FINDINGS AND PROPOSED COURSE: One contract laboratory, Southern Research Institute, has served as a model for TDMS implementation. All of the bioassay studies there are on-line; the data is being captured on terminals and transmitted to the mainframe computer at NCTR. With the assistance of Southern Research Institute systems development for animal room data, toxicology data, and pathology have been completed and can now be used by other contract labs. Reports suitable for contract lab usage and others designed for NIEHS usage (summaries of the data) have been developed and verified. Suitable storage and retrieval, verification, and user authorization systems for the computer-stored data have been developed.

Four other contract laboratories, Battelle-Columbus, Microbiological Associates, E.G. and G. Mason, and International Research and Development Corp., have been selected for TDMS and are using manual forms to facilitate computer entry when appropriate. These labs have now received the necessary hardware and will begin automated data entry this fiscal year. As chronic starts ensue at the remaining contract labs similar steps for automation will be employed.

Additional hardware for contract labs and for NIEHS have been ordered. Installation of this equipment will permit direct data access by NIEHS scientists and communication between NIEHS and contract labs. A query language processor system is proposed to enable NIEHS to examine the data arranged in various desired formats.

Successful completion of the above phases will enable NIEHS to collect all types of prechronic data at all of the laboratories.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:
Implementation of TDMS will permit rapid management decisions for contract operations, improve the accuracy and uniformity of data collection, and enable better comparisons with a historical data base of toxicology and carcinogenicity. These improvements will enhance the quality of the data on each chemical tested that will ultimately be utilized as guidelines for evaluating potential human risk.

BATTELLE COLUMBUS LABORATORIES - Columbus, Ohio 43201
NO1-ES-8-2151

TITLE: Comparative Carcinogenicity and Toxicity Studies of Selected Environmental Chemicals in Laboratory Animals Exposed During Pre- and Postnatal Life

CONTRACTOR'S PROJECT DIRECTOR: Arthur C. Peters, D.V.M.

PROJECT OFFICER (NIEHS): Rajendra S. Chhabra, Ph.D., Supervisory Pharmacologist, Toxicology Research and Testing Program (TRTP)

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $949,974

PROJECT DESCRIPTION

OBJECTIVES: The main objective of this study is to determine if there is increased sensitivity of laboratory animals to potential carcinogenic and toxicologic effects of selected environmental chemicals exposed at various dose levels during their in utero development plus postnatal life of two years as compared to the animals exposed only during post-weaning time of two years. This objective will be achieved by (1) studying the carcinogenic potential of selected environmental chemicals exposed to the laboratory animals during their in utero development and weaning period (by treating their mothers with the chemical until weaning) followed by life time exposure (2 years) and comparing it with the effects observed in parallel groups of animals (derived from untreated mothers) exposed to the chemicals only after weaning for two years; (2) studying the effects on reproduction, behavioral, endocrine, and immunological functions at specific time periods during the bioassay in additional groups of animals (incorporated in the bioassay design); and (3) the bioassay will be carried out at 3 dose levels plus controls to determine if there is a dose-response relationship of the chemical for carcinogenic and other toxicologic endpoints.

METHODS EMPLOYED: Under this contract three chemicals are being studied in both male and female B6C3F1 mice and F344 rats. The project is divided into two phases, i.e. prechronic and chronic.

Prechronic Phase of Study: The acute toxicity and MTD (Maximum Tolerated Dose) determination is carried out by following the "Guidelines for Carcinogenic Bioassay in Small Rodents" prepared by the Division of Cancer Cause and Prevention, NCI, NIH.

The Maximum Neonatal Dose (MND) will be determined as follows: mature females (7-9 weeks) will be divided into five groups, i.e. (1) control, (2) MTD (as determined in 90 day studies), (3) 1/3 MTD, (4) 1/10 MTD, and (5) 1/30 MTD. Twelve females per group will be dosed with the chemical (in diet) before breeding. These groups will be bred to untreated, proven fertile males after four weeks or to time of steady state for the chemical and continued on the chemical through weaning of the F_1 generation. The size of litters will be limited to 8 in rats and 6 in mice by random killing of excessive animals on day 4. The MND will be the highest dose at weaning which does not depress the body weight of the offspring more than 10% as compared to the controls; and does not produce mortality, clinical signs of toxicity, pathologic lesions or malformations that would be

predicted to shorten the animals' natural life span. Extra animals will be added in MND determination experiments to analyze the chemical and/or its metabolite(s) concentrations in the tissues and body fluids by using standard analytical GLC or mass spectrometry methods.

Chronic Phase: In addition to the development of tumors as an end point, the appropriate toxicity tests, general histopathology, behavioral and immunology function tests will also be performed on parallel sets of animals placed on same dose regimen at specific time intervals during the test period. The chronic phase will begin with 4 groups of sexually matured (7-9 weeks) females of both species; groups of 90 animals will receive MND of the chemical; two groups of 30 animals in each will receive 1/3 MND and 1/10 MND respectively; fourth group of 90 animals will not receive any treatment. The dosing will begin 4 weeks later, or to the time of steady state for the chemical, before breeding of all groups. Three days before anticipated delivery, the animals will be transferred to a suitable cage to litter. The F_0 females will continue to receive the test chemical while nursing their litters.

A. Carcinogen Bioassay - At weaning of above four groups, not more than 2 males and 2 females, shall be selected randomly from each litter to obtain the total required for the carcinogen bioassay. Eight groups (16 for both sexes) consisting of 50 offspring in each (derived from F_0 mothers) will be treated with test chemical for 2 years as outlined below.

F_0 Treatment Group	F_1 Offspring Randomized Grouping	F_1 Treatment
	———	MTD
MND	———	1/3 MTD
	———	No treatment
1/3 MND	———	1/3 MTD
1/10 MND	———	1/10 MTD
	———	MTD
untreated	———	1/3 MTD
	———	Control

For evaluation of carcinogenic potential the contractor will follow specific toxicopathologic procedures suggested by NIEHS.

B. General Toxicology Tests - A number of tests will be performed on separate animals incorporated in the carcinogen bioassay design. These animals will be exposed to the test chemical at the same dose regimen as that of carcinogen bioassay groups. Various toxicologic endpoints to be tested are described below.

I. Toxico-Pathologic Evaluation - A parallel set of 8 groups of each sex shall be set up. These groups will consist of 10 male and 10 female animals at each test level. Each group shall consist of one F_1 male

and one F_1 female randomly selected from each 10 litters. These groups will be placed on the appropriate treatment at weaning and sacrificed at 9 months of age for toxico-pathologic evaluations which include gross pathology, histopathology, clinical chemistry and tissue levels of the test chemical.

II. Reproductive Function Tests - The animals of the Toxico-Pathologic group, prior to their sacrifice, will be subjected to reproductive function tests.

III. Immune-Function Tests - One additional set of eight groups will consist of 12 males at each test level. Each group will consist of one F_1 male randomly selected from each of 12 litters at weaning and placed on the appropriate treatment. The animals will be sacrificed at 9 months for immune-function tests such as: T-cell function, in vitro by assessing the response of splenic or peripheral blood lymphocytes to mitogen conconavalin A and/or phytohemagglutinin; in vitro B-cell function by assessing its in vitro response of lymphocytes to poke weak mitogen or E. Coli lipopolysaccharide; antibody response to T-dependent antigen by plaque assay; delayed hypersensitivity reaction; and quantitation of immunoglobulin.

IV. Behavioral Tests - A battery of behavioral tests will be applied to 10 male rats per treatment groups from 10 separate litters and 20 male controls from separate litters. The behavioral tests will be performed in the same animals at 4 weeks, 9 months, and 2 years of carcinogen bioassay groups. The tests will include spontaneous motor activity; presence or absence of autonomic signs and for the appearance of normal or deferred motor and pain reflexes; visual placement responses; forelimb grip strength; hind limb extensor reflexes; startle responsiveness and habituation to a time-locked acoustic signal; and one-way avoidance response.

MAJOR FINDINGS AND PROPOSED COURSE: There were originally four chemicals, i.e. Phenytoin, Ethylenethiourea, Firemaster FF-1 and Kepone, that were selected for study under this contract. However, due to the budgetary constraints, Kepone was withdrawn from this study. The following is the status of study on the individual chemicals:

Phenytoin: All pre-chronic studies have been completed. The histopathologic examination of tissues from MND experiments will be finished soon. The results from the subchronic toxicity study have shown some species differences in toxic response and these results were presented at the Society of Toxicology Annual meeting. The chronic phase of this study was initiated during this reporting period.

Ethylenethiourea: In the subchronic toxicity study the compound-induced histomorphologic lesions were found in the oesophagus, liver, and thyroid of the mice. The thyroid hyperplasia found in the two highest dosages were seen as a preneoplastic response to ETU exposure. In rats, compound-induced histomorphologic lesions were found in the bone marrow, esophagus, liver, pituitary, nonglandular portion of the stomach and thyroid. The thyroid adenomas were seen in the high dosage groups in both sexes.

The MND studies have been extended to 9 weeks for this compound to determine if the carcinogenic potential of this chemical and can be detected at an early age. The data from MND studies is being evaluated for selection of dose levels for the chronic phase. The chronic phase will be initiated during this fiscal year.

Firemaster FF-1: The facilities modification for performance of the PBB study has been completed. The facilities have been inspected by the NTP representatives and were found to be generally satisfactory but needed some minor adjustments. The MND studies have been completed. The histopathological evaluation is underway. The chronic phase will begin during November, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The discovery of cancer in the daughters of women exposed to diethylstilbesterol, toxicity in babies exposed to hexachlorophene, or morphologic and functional impairments in children whose mothers were exposed to environmental mercury emphasizes the significance of prenatal exposure to chemicals and resulting delayed toxicologic or carcinogenic effects in offspring. The objective of this program is to test the concept that prenatal plus postnatal exposure of chemicals for carcinogen bioassay is perhaps a more sensitive method for assessment of toxicity and carcinogenicity of selected chemicals as compared to widely used approach of lifetime exposure of young adult animals. This research project is directly relevant to the mission of the National Toxicology Program implemented by NIEHS. The data from this research will aid in better risk assessment of the selected chemicals for human exposure for a wide spectrum of the population as well as strengthen the need for different approaches necessary for recognition of reversible or irreversible toxic properties of chemicals in general or for a class of chemicals.

PUBLICATIONS

Kurtz, P., Peters, A., Donofrio, D. and Chhabra, R.: Species differences in Diphenylhydantoin (Phenytoin) toxicity. The Toxicologist, 1: 114, 1981.

Kurtz, P., Peters, A., Donofrio, D. and Chhabra, R.: Subchronic toxicity of ethylenethiourea in mice and rats. The Toxicologist, 2: 342, 1982.

Chin, A. E., Kurtz, P. J., Calton, B. D., Peters, A. C. and Chhabra, R. S.: The disposition and metabolism of diphenylhydantoin in maternal, fetal and neonatal tissues after perinatal exposure of rat dams. The Toxicologist, 2: 437, 1982.

SOUTHERN RESEARCH INSTITUTE
Birmingham, Alabama 35255
(NIH-NO1-CP-95651-02)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for Polysorbate 80, Ethylene Glycol, CI Pigment Red 3, and CI Pigment Red 23

CONTRACTOR'S PROJECT DIRECTORS: Dr. J.D. Prejean, Head
Dr. D.P. Griswold

PROJECT OFFICER (NCI/NTP): William C. Eastin, Ph.D., Physiologist, Carcinogenesis and Toxicological Evaluation Branch, NTP

DATE CONTRACT INITIATED: September 30, 1980

CURRENT ANNUAL LEVEL: $473,907

PROJECT DESCRIPTION

OBJECTIVES: The objective of this program is to investigate the carcinogenicity of four selected chemicals using Fischer-344 rats and B6C3F1 mice. The protocol consists of a series of prechronic studies to determine a maximum tolerated dose (MTD) for use in a chronic study in which the MTD and MTD/2 are administered to 50 animals/sex/species/dose level for 103 weeks followed by a one-week observation period. All chemicals are being administered by dosed-feed and the appropriate untreated controls are included with each study. All of the animals assigned to a chronic study undergo complete necropsy and approximately 42 tissues are evaluated histopathologically for evidence of tumors or non-tumor pathology. In addition, the subchronic study protocols for ethylene glycol, CI Pigment Red 3, and CI Pigment Red 23 were expanded to include supplemental studies such as urinalyses, electrolyte assays, and enzyme profiles.

METHODS EMPLOYED: The toxicity and carcinogenicity studies are performed in accordance with the requirements in the Basic Ordering Agreement for the NTP Bioassay Contract. Hematology, urinalysis, and clinical chemistry studies were performed during bioassay of ethylene glycol, CI Pigment Red 3, and CI Pigment Red 23. The Toxicology Data Management System was used to collect subchronic data for both Pigments Red 3 and 23. This system will be used to capture data in the chronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: Prechronic testing of polysorbate 80, ethylene glycol, CI Pigment Red 3, and CI Pigment Red 23 has been completed. Prechronic reports were submitted for polysorbate 80 and ethylene glycol and these are in review by the appropriate NTP chemical managers. Histopathology for CI Pigments Red 3 and 23 is in progress. During 1982 the contractor proposes to complete the prechronic phase for Pigments Red 3 and 23 including submission of prechronic reports to the NTP. They further propose to initiate chronic studies for polysorbate 80 and ethylene glycol as directed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Results of these studies will provide information about the carcinogenicity of each chemical in experimental animals which can be used as a guideline for evaluating their potential hazard to man.

SOUTHERN RESEARCH INSTITUTE
Birmingham, Alabama 35255
(NIH-NO1-CP-95651-01)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for Furan, Furfuryl Alcohol, Furfural, Gamma-Butyrolactone, Benzaldehyde, and Hexachlorocyclopentadiene

CONTRACTOR'S PROJECT DIRECTORS: Dr. J.D. Prejean, Head
Dr. D.P. Griswold

PROJECT OFFICER (NCI/NTP): William C. Eastin, Ph.D., Physiologist, Carcinogenesis and Toxicological Evaluation Branch, NTP

DATE CONTRACT INITIATED: June 30, 1980

CURRENT ANNUAL LEVEL: $804,203

PROJECT DESCRIPTION

OBJECTIVES: The objective of this program is to investigate the carcinogenicity of six selected chemicals using Fischer-344 rats and B6C3F1 mice. The protocol consists of a series of prechronic studies to determine a maximum tolerated dose (MTD) for use in a chronic study in which the MTD and MTD/2 are administered to 50 animals/sex/species/dose level for 103 weeks followed by a one-week observation period. All chemicals are being administered by gavage and the appropriate vehicle controls are included with each study. Al of the animals assigned to a chronic study undergo complete necropsy and approximately 42 tissues are evaluated histopathologically for evidence of tumors or non-tumor pathology. In addition, the standard subchronic protocol on furan, furfuryl alcohol, and furfural was expanded to include a series of special studies designed to identify the metabolic products of each chemical.

METHODS EMPLOYED: The toxicity and carcinogenicity studies are performed in accordance with the requirements in the Basic Ordering Agreement for the NTP Bioassay Contract. Special studies for the metabolism of furan, furfural, and furfuryl alcohol were performed. The Toxicology Data Management System will be used to capture data in the chronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: The acute, repeated dose, and subchronic studies on furan, furfuryl alcohol, furfural, gamma-butyrolactone, benzaldehyde, and hexachlorocyclopentadiene (HCCP) were completed. Special studies indicate that furfural and furfuryl alcohol are metabolized by the same pathway. In addition, a special study done elsewhere showed that the best route of exposure for HCCP is inhalation. Therefore, it was decided not to continue with furfuryl alcohol and HCCP as designed into the chronic phase. These two chemicals have been deleted from this contract. During 1982 the contractor will continue with the chronic studies on gamma-butyrolactone, benzaldehyde, furfural, and furan.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Results of these studies will provide information about the carcinogenicity of each chemical in experimental animals which can be used as a guideline for evaluating their potential hazard to man.

TEMPLE UNIVERSITY SCHOOL OF MEDICINE
Philadelphia, Pennsylvania 19140
(NIH-NO1-CP-15752)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. P. Donald Forbes

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $200,000

PROJECT DESCRIPTION

OBJECTIVES: This contract is designed to investigate the toxicity and/or carcinogenicity of the psoralens with and without uv light. This project will test a variety of promising psoralen compounds and compare the relative toxicities. The toxicity of 8-methoxypsoralen (8-MOP), 3-carbethoxypsoralen (3-CEP), 5-methylisopsoralen (5-MIP) and 5-methoxypsoralen (5-MOP) will be studied in the HRA/skh mouse. This contractor will provide HRA/skh mice to the other NTP contractors involved in the psoralen project.

METHODS EMPLOYED: Animal studies; pathological analysis of tumors; maintenance of animal colony.

MAJOR FINDINGS AND PROPOSED COURSE: This contractor has begun a 13-week study in the HRA/skh mouse to compare the toxicologic properties of 8-MOP, 3-CEP, 5-MIP and 5-MOP. Each compound is being tested with and without UVA light. The laboratory phase of this study will be completed on August 10, 1982 and then the pathology assessment will be performed. The NTP plans to initiate a chronic study with 8-MOP in the HRA/skh mouse.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus UVA light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

BIOASSAY SYSTEMS CORPORATION
Woburn, Massachusetts 01801
(NIH-NO1-CP-15753)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Kenneth S. Loveday

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $157,501

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to investigate the toxicity and/or carcinogenicity of the psoralens with and without uv light. The contractor will test a variety of promising psoralen compounds and compare the relative toxicities. The metabolism of 8-methoxypsoralen (8-MOP), 3-carbethoxypsoralen (3-CEP), 5-methylisopsoralen (5-MIP) and 5-methoxypsoralen (5-MOP) in the HRA/skh mouse will be studied. The ability of the four psoralen compounds to induce mutations will be determined.

METHODS EMPLOYED: Tissue distribution of psoralens in the HRA/skh mouse; short term in vitro tests.

MAJOR FINDINGS AND PROPOSED COURSE: Metabolism studies - Tritium labelled 8-MOP has been given by gavage to HRA/skh mice. Three animals were sacrificed per time period at 0, 15, 30, 60 minutes and at 2, 4, 8, 24, 72 and 96 hours. The distribution of 8-MOP was determined, and after 30 min. the highest concentrations were seen in the liver, kidney and blood. Metabolites in the urine will be fingerprinted by high pressure liquid chromatography. Genetics studies - Bioassay Systems is investigating the in vitro and in vivo induction of mutations and sister chromatid exchanges using the CHO cell line. These studies include the affects of psoralens with and without UVA. The psoralens being studied include 8-MOP, 5-MOP, 3-CEP and 5-MIP. Two papers on these topics will be published in the Journal of the National Cancer Institute.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus UVA light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

HRI ASSOCIATES
Emeryville, California 94608
(NIH-NO1-CP-15756)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Drs. John E. Hearst/Stephen Isaacs

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this contract was to test the interaction of DNA with 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), 3-carbethoxy-psoralen (3-CEP) and 5-methylisopsoralen (5-MIP).

METHODS EMPLOYED: Radiolabelling of psoralens using tritiated methyliodide; spectral characterization of psoralens (mass-spectral analysis, NMR spectral analysis); chromotographic characterization of psoralens (thin-layer chromatography and high performance liquid chromatography); determination of DNA binding using radiolabelled psralens and calf thymus DNA.

MAJOR FINDINGS AND PROPOSED COURSE: This contract has completed its work (March 31, 1981-March 30, 1982) and the findings are as follows:

The four psoralens were successfully radiolabelled and these radiolabelled compounds were used to determine the binding to DNA in the dark and in the presence of UVA light. In the dark 8-MOP, 5-MOP and 5-MIP bind to calf thymus DNA while 3-CEP does not. All compounds bind to DNA in the presence of UVA, but 3-CEP shows the slowest binding. The peak binding levels expressed as psoralen molecules per DNA base pairs are: 5-MOP (37 Ps/1000 base pairs); 5-MIP (32 Ps/1000 base pairs); 8-MOP (31 Ps/1000 base pairs) and 3-CEP (1 Ps/1000 base pairs).

The DNA unwinding angles for each psoralen were also determined. The details of this work are described in "In Vitro Characterization of the Reaction of Four Psoralen Derivatives with DNA", a paper to be published in JNCI.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus UVA light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. The information on psoralen-DNA interactions will be used to help explain the mechanism of action for PUVA therapy, and to develop new and better psoralen derivatives. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

EMORY UNIVERSITY/VA HOSPITAL
Atlanta, Georgia 30322
(NIH-NO1-CP-15767)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Isaac Willis

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $20,000

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to compare the relative toxicity of 8-methoxypsoralen (8-MOP) and 5'-aminomethyl-4,4'8-trimethylpsoralen (5'-AP) in HRA/skh mice. These studies will be conducted with and without uv light.

METHODS EMPLOYED: animal studies with HRA/skh mice; pathology/histopathology

MAJOR FINDINGS AND PROPOSED COURSE: This contractor has demonstrated that 5'-AP, a water soluble psoralen, gives a shorter time to peak response (as measured by skin reactions) than does 8-MOP. In addition, 5'-AP is a more potent oral photosensitizer, a property which has been equated with better therapeutic potential. This group reports that 5'-AP has low systemic toxicity. The results of these studies are summarized in the report entitled "Psoralens: A Search for More Effective Derivatives for Photochemotherapeutic Regimens", in preparation for JNCI. This project is now completed and further studies with 5'-AP are not anticipated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus UVA light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

UCLA SCHOOL OF MEDICINE
Los Angeles, Califonia 90024
(NIH-NO1-CP-15768)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Nicholas J. Lowe

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $20,000

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to investigate the toxicity and/or carcinogenicity of psoralen compounds with and without uv light. This contractor will test 8-methoxypsoralen (8-MOP), 3-carbethoxypsoralen (3-CEP), 5-methylisopsoralen (5-MIP), and 5-methoxypsoralen (5-MOP). These compounds will be tested in the Salmonella lambda prophage induction test. The effects on psoralens on enzyme activities in HRA/skh mouse skin will be measured.

METHODS EMPLOYED: Short term in vitro assays; enzyme assays (ornithine decarboxylase and S-adenosyl-1-methionine decarboxylase).

MAJOR FINDINGS AND PROPOSED COURSE: This contractor has demonstrated that 8-MOP and 5-MOP increase the levels of epidermal ornithine decarboxylase and decrease epidermal DNA synthesis. 3-CEP does not show these effects. An increase in epidermal ornithine decarboxylase has been associated with tumor promoting agents. 8-MOP and 5-MOP have also been shown to be inducers of prophage in the Salmonella TA 1535 and TA 1538 strains. Studies on the effects of psoralens on S-adenosyl-1-methionine will be done during the coming contract year. 5-MIP has just recently been synthesized and this compound will be tested in all assay systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralens plus UVA light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. The results of this contract will be related to the results of the chronic toxicity studies in the HRA/skh mouse. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

SMALL BUSINESS ADMINISTRATION - DINGLE ASSOCIATES
Washington, D.C. 20006
(SB-4-0-8[a]; 81-C-1488)

TITLE: Conference on Clinical and Experimental Aspects of Psoralen Toxicology

CONTRACTOR'S PROJECT DIRECTOR: Mr. Ramsey L. Sa'di

PROJECT OFFICER (NIEHS): June K. Dunnick, Ph.D., Chemist, Carcinogenesis and Toxicology Evaluation Branch, TRTP

DATE CONTRACT INITIATED: April 30, 1981

CURRENT ANNUAL LEVEL: $64,975

PROJECT DESCRIPTION

OBJECTIVES: The contractor will provide support services for the National Toxicology Program Conference on "Photobiologic, Toxicologic and Pharmacologic Aspects of Psoralens" held March 1-3, 1982 at NIEHS, Research Triangle Park, NC.

METHODS EMPLOYED: Support services for travel arrangements, typing agendas and other conference arrangements.

MAJOR FINDINGS AND PROPOSED COURSE: This contractor successfully arranged for 30 participants to attend the NTP Psoralen Conference on March 1-3, 1982 in the Research Triangle Park, NC. This included arranging and paying for travel and room expenses. In addition, this contractor provided other support for the conference including preparing a printed agenda and a transcript of the meeting. The services provided by this contract were essential to the success of the meeting.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The NTP Psoralen Conference brought together 96 scientists from the United States and Europe to discuss toxicologic properties of ultraviolet light and psoralen. This conference discussed the mechanisms involved in uv/chemical toxicity, and the relationship of DNA damage to cancer development. The proceedings of this conference will be published as a monograph by the Journal of the National Cancer Institute.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE - Chicago, Illinois 60616
NIEHS-NO1-ES-5-2156

TITLE: Long Term Study on the Effect of Ingested Asbestos in Hamsters

CONTRACTOR'S PROJECT DIRECTOR: Alan M. Shefner, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: This contract is for the purpose of studying the long term effects of ingestion (via feed) of asbestos in hamsters. Types of asbestos fibers being studied are short range (fiber size) chrysotile, intermediate range chrysotile and amosite. In addition, low levels of 1,2-dimethylhydrazine (a known intestinal carcinogen) are being used in conjunction with intermediate range chrysotile to study its co-carcinogenic potential.

METHODS EMPLOYED: The above asbestos fibers are mixed in the food at the rate of 1% in the diet and the male and female hamsters are fed this diet for their lifetime. Parameters evaluated were body weight gain, clinical effects, and most importantly the macro- and histopathology observed at death.

MAJOR FINDINGS AND PROPOSED COURSE: All hamsters have died and the histopathology has been completed. Results showed that the various forms of asbestos did not affect weight gain, survival or cause neoplasia. This project is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas in the United States are contaminated with asbestos fibers as are several common food items. Because of this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

PUBLICATIONS

McConnell, E.E., Haseman, J.K., and Moore, J.A.: NTP technical report on the carcinogenesis bioassay of amosite asbestos in Syrian golden hamsters. NTP-81-58, in press.

McConnell, E.E., Haseman, J.K., and Moore, J.A.: NTP technical report on the carcinogenesis bioassay of chrysotile asbestos in Syrian golden hamsters. NTP-81-51, in press.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 21180
NIEHS-NO1-ES-5-2158

TITLE: Long Term Study on the Biological Effects of Ingested Asbestos in Rats

CONTRACTOR'S PROJECT DIRECTOR: Henry A. Rutter, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: This contract is for the purpose of studying the long term effects of ingestion (via feed) of various types of asbestos fibers and tremolite in rats. The types of asbestos fibers being studied are intermediate range chrysotile, short range chrysotile, crocidolite and amosite. In addition, low levels of 1,2-dimethylhydrazine (DMH) (a known intestinal carcinogen) are being used in conjunction with amosite and intermediate range chrysotile to study its co-carcinogenic potential.

METHODS EMPLOYED: The above fibers were mixed in the food at a rate of 1% in the diet and the male and female rats are fed this diet for their lifetime. Parameters being evaluated are body weight gain, clinical effects and most importantly the macro- and histopathology observed at death.

MAJOR FINDINGS AND PROPOSED COURSE: As of May 1, 1982 all of the rats on the study have died and the histopathology has been completed. The survival of all asbestos groups (except the groups with DMH) were comparable to the controls. In other words, no life-shortening effects were observed. The original pathologists findings are currently undergoing review by the NTP Pathology Working Group. Final data on the various types of fibers will be completed in late FY 82 and FY 83.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas of the United States are contaminated with asbestos fibers as are several common food items. Because of this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

CELLULAR AND GENETIC TOXICOLOGY BRANCH

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The goal of the Cellular and Genetic Toxicology Branch of the Toxicology Research and Testing Program is to provide an integrated research and testing program using short-term test systems to evaluate the genetic toxicity of selected chemicals. The program is also aimed at understanding the mechanisms of cellular and genetic toxicity in order to provide a basis for further test system development, and interpretation of test results. Emphasis is currently placed on short-term tests that measure mutagenicity and aneuploidy in microbial cells as well as mutagenicity, DNA and cytogenetic damage, and oncogenic transformation in mammalian cells. Test data are used to set priorities for further testing and in the design and interpretation of long-term animal carcinogenicity, mutagenicity, and toxicity studies.

An implicit goal of the cellular and genetic toxicology effort is to establish a scheme of short-term testing which can be used to predict chemical carcinogenicity and mutagenicity and thereby reduce the need for in vivo assays or assist in setting testing priorities for long-term animal bioassays. However, for short-term tests to be predictive, several criteria must be fulfilled which include both a knowledge of the reproducibility of individual test results and the relationship of the endpoint measured to carcinogenicity, mutagenicity, or other in vivo toxic effects. The application of a group of complementary tests which meet these criteria should ultimately result in an effective system for testing chemicals. An important part of the Branch program is to produce sufficient short-term test data, particularly across chemical classes, to relate short-term test results to carcinogenic effects in animals and man. Even with the appropriate use of available test systems, some potential carcinogens (or cocarcinogens or tumor promotors) may not be identified, particularly those that do not induce damage leading to observable gene mutations or chromosomal changes. It is therefore important that we continue to develop new methods capable of detecting carcinogens not identified by the assays currently in use and to distinguish chemicals which "promote" tumor development. In order to accomplish these goals, it is important that the program remain involved in and responsive to basic research developments together with their potential applications.

A substantial portion of Branch resources are committed to studies of chemically induced mutations. These mutation studies can be divided into two categories: 1) somatic mutation studies; and 2) germinal (gonadal) mutation studies. The major difference between the two is that mutations arising in germinal cells can be transmitted to subsequent generations, while somatic mutations can only be expressed in the affected individual. Mutagenicity testing systems are designed to measure either somatic or germinal mutations; in vitro test systems are considered relevant for somatic mutation because they measure mutagenicity in mitotically dividing cells. However, the information gained from tests using in vitro systems also has implications for heritable mutation risk because a chemical that is mutagenic in vitro has the potential to be mutagenic in gonadal cells in vivo. (By the same argument, germ cell mutagens are likely to be somatic cell mutagens.)

The portion of the program concerned with assay validation and testing is performed through extramural contracts, while basic research, test development or modification, and data management and analysis activities are generally performed intramurally. Intramural research efforts involve both prokaryotes

and eukaryotes. Studies on the Salmonella typhimurium mutation tester strains include attempts to increase our understanding of the test system, to improve the sensitivity and efficiency of protocols currently in use and to use the Salmonella test as a tool to study in vitro and in vivo metabolism of mutagenic chemicals. Studies with the yeast Saccharomyces cerevisiae are directed at the meiotic process in an effort to better understand the genetic events of meiosis at molecular and enzymological levels; to determine the role of DNA repair mechanisms in mutational processes and to understand meiotic and mitotic recombination and DNA repair processes. Studies in Drosophila melanogaster are directed at characterization of the relationship between DNA repair and mutagenesis using X-linked mutagen-sensitive mutants, many of which are defective in DNA repair. Such repair-defective mutants have also been combined with certain naturally occurring transposable elements to allow the study of interactions that occur in double mutant combinations. Alterations in gene structure and expression which may result from chemical and physical toxic/carcinogenic agents are also being analyzed in mammalian cells by recombinant DNA techniques with particular attention to potential gene or sequence transpositions. Finally, a new project has been added that is directed at the organ specificity of chemical carcinogens. Using liver and bladder cells, organ and species specificities of the chemical activation of several carcinogen classes are being investigated. Details of these respective projects are described in the accompanying project reports.

The key extramural contract activities include microbial, Drosophila, and mammalian cell mutagenesis testing; in vitro cytogenetic testing (chromosome aberrations and sister chromatid exchanges) and the development and evaluation of an in vivo mouse assay for chemically induced cytogenetic damage; development and validation of a multiple endpoint mutation system in cultured mammalian cells; the development of assays for induction of aneuploidy in Drosophila and in yeast; in vitro assay in mammalian cells for induced DNA damage; a dual laboratory coded compound evaluation of three mammalian cell transformation systems including Syrian hamster embryo (SHE) cells; SHE cells infected with Simian adenovirus (SA7), and retrovirus infected rat cells (both of which measure chemical enhancement of viral transformation); the final phase of evaluation of Balb/c 3T3 cells for chemical transformation is in progress.

A coordinated project effort was initiated in FY 1981 to test for the genetic toxicity of 19 chemicals which are priority candidate bioassay chemicals for 1982. Other developmental projects include an effort to develop a standardized protocol by which the frequencies of chromosome aberrations and sister chromatid exchanges can be accurately and reproducibly measured in human lymphocytes with particular emphasis on understanding the sources of variation that may affect the measurement of these endpoints. Efforts to measure germinal mutations in mice involve the morphological specific locus assay and the search for electrophoretic variant isozymes which result from specific chemical exposures. Other developmental projects include identification of mutagens produced in cooked foods and an attempt to develop an assay for specific sequence transpositions in mammalian cells.

In summary, the Branch is directly involved in basic research on mutagenesis and related genotoxicity processes, improvement and validation of short-term tests for mutagens and carcinogens, and the application of tests designed to detect and characterize chemicals that may pose carcinogenic or genetic risks to humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21012-01 CGTB

PERIOD COVERED
February 21, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Organ and Species Specificities in Chemical Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Langenbach	Microbiologist	CGTB	NIEHS
	James Allen	Microbiologist	GTD	EPA

COOPERATING UNITS (if any)

Linda Oglesby, Northrop Services, Inc.

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An in vitro approach for studying the organ, species, strain and individual variations in the activation of chemical carcinogens has been developed. Rat, mouse, hamster, bovine, and canine species have been used extensively with liver and bladder being the primary organs studied. Both intact cells and cell homogenates have been used for activation. To assess biological activity, the multiple genetic endpoints of toxicity, SCE and mutation induction in V79 cells and/or reversion of Salmonella typhimurium are used. Species, strain and organ differences have been observed for chemicals such as nitrosamines, aromatic amines, hydrocarbons, and carbamate derivatives. In general, the relative mutagenic or cytogenetic activity correlates with the in vivo carcinogenic activity.

METHODS EMPLOYED: The target organism, V79 cells or S. typhimurium, are co-cultivated with intact bladder or liver activating cells or incubated with the cell homogenates (S-9). Cytotoxicity, SCE and mutation induction are measured in V79 cells and reversion in s. typhimurium as the genotoxic endpoints.

MAJOR FINDINGS AND PROPOSED COURSE: The most significant recent accomplishments are the development of a bovine bladder cell and S-9 mediated S. typhimurium and V79 cell mutagenesis systems. For noninbred animals, individual variations in bladder cell activation between animals has been well documented. Bladder cells are 10 to 50 fold more active than liver cells at producing mutagens from aromatic amines, but are less active than liver cells for nitrosamines and hydrocarbons. The system discriminates between the bladder carcinogens and noncarcinogens. Additionally, it has been observed that the endpoint studied can influence the observed genotoxic activity of the chemical. For example, hydrocarbons and nitrosamines are most sensitively detected with V79 cells as the target while S. typhimurium is substantially more reliable for aromatic amines. Future work will probe the possible interactions of the bladder and liver in the metabolic activation of bladder carcinogens. Also human tissues will be utilized so that the effect of suspect chemicals on human bladder cancer initiation can possibly be more directly determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The studies provide a methodology for evaluating one major component known to determine organ and species specificity. Such data will aid in elucidating the mechanism of bladder cancer initiation and hopefully facilitate extrapolation of bladder bioassay data to human hazard.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21013-01 CGTB

PERIOD COVERED
November 29, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Lawrence R. Boone Senior Staff Fellow CGTB NIEHS
Raymond W. Tennant Chief, CGTB CGTB NIEHS

COOPERATING UNITS (if any)

Wen K. Yang Biology Division, ORNL (Contract Y01-ES-10061)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to analyze by recombinant DNA techniques sequence transpositions and alterations in gene structure and expression which result from chemical and physical toxic/carcinogenic agents. Molecular clones of the "transposon-like" endogenous ecotropic viral genomes of BALB/c and RFM/Un mice have been constructed and characterized. Subgenomic regions have been subcloned to provide molecular probes to specifically detect the ecotropic provirus and any long terminal repeat (LTR) containing genetic regulatory elements. Detailed analysis of a large population of cloned genomes of the BALB/c endogenous retrovirus has revealed a significant variability in the U3 region of the LTR. These findings are intriguing due to the important multifunctional role of this region, including "transposon-like" integration and regulation of gene transcription. Ongoing and future studies involve the molecular cloning of reintegrated or transposed proviral genomes when identified in cultured and primary radiation induced myeloid leukemia cells. Analysis of alterations in gene expression due to the relocation of these elements will be performed using cloned DNA probes from adjacent cellular regions.

PHS-6040
(Rev. 2-81)

OBJECTIVES: The long range goal of this project is to clarify the role which chemical and physical environmental agents have in initiating the transposition or structural alteration of genes and regulatory elements and how these events result in genetic toxicity. An immediate goal is to identify and characterize transpositions of genetic elements which may be casually related to radiation induced myeloid leukemia. The initial model will be based on the "transposon-like" endogenous provirus of RFM/Un mice.

METHODS EMPLOYED: Recombinant DNA techniques will be used to generate molecular probes from endogenous retroviral genomes. Primary (germ line provirus) and secondary (transpositions and reintegrations) loci will be molecularly cloned from normal cells and radiation induced myeloid leukemia cells. Restriction endonuclease mapping, nucleic acid sequencing and characterization of specific RNA transcript will be employed.

MAJOR FINDINGS AND PROPOSED COURSE: Recombinant DNA clones of the endogenous ecotropic viral genome of BALB/c RFM/Un mice have been constructed and characterized. Subgenomic regions have been subcloned to provide molecular probes to specifically detect the ecotropic env gene and the viral LTR. Detailed mapping of the LTR region of a population of clones has revealed that a particular segment of the "U3" component of the LTR appears to be a 'hyper variable' region. The LTR is known to be involved in "transposon-like" integration and regulation of viral gene transcription and promotion of transcription of adjacent cellular genes. Continued work is aimed at the mechanism of generating this diversity and its biological significance. Ongoing and future studies will focus on the molecular cloning of the primary and secondary loci and characterization of gene expression patterns in adjacent regions in normal and neoplastic cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The mechanism of chemical or physical genetic toxicity/carcinogenesis by gene rearrangement and/or structural alterations and how these events relate to normal cell differentiation processes is of considerable importance in contemporary biomedical research. The ability to detect such events in a sensitive and precise manner, and the ability to identify chemical and physical agents with the potential to cause such effects, are fundamental goals of the CGTB/NTP/NIEHS. This project is aimed at providing basic information concerning those processes and on which reliable assay systems can be based.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21014-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mathematical Modeling of DNA Repair and Recombination in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Resnick	Research Geneticist	CGTB	NIEHS
	T. Darden	Staff Fellow	BRAP	NIEHS

COOPERATING UNITS (if any)

Biometry and Risk Assessment Program

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetic recombination is an essential feature of normal meiosis and during repair of various types of DNA damage. As part of a program to understand the mechanisms of recombination and its genetic control, the timing and the location of recombination events are being evaluated by applying modeling procedures to biochemical data. DNA lesions are used as markers in exchange processes where the lesions can be identified using enzymes which will nick the DNA, and thus reduce the size of the DNA. If recombination occurs, lesions induced in parental strands of DNA can become associated with newly synthesized DNA; therefore, as a result of recombination events, newly synthesized DNA can become sensitive to nicking enzymes. Using this approach, we have been able to predict, by mathematical modeling, the detectibility of exchanges knowing the average number of lesions per parental molecule. Analysis of results with E. coli indicates that every unrepaired dimer (induced by UV) causes a reciprocal exchange event. Results with yeast undergoing meiosis or during mitotic growth indicate that DNA damage is very inefficient at causing reciprocal exchange events; similar conclusions can be drawn for mammalian cells.

METHODS EMPLOYED: Exchanges of DNA in which parental and newly synthesized strands can be distinguished lead to covalent linkage of parental and new strands. If DNA damage is present in the parental strands and this damage can be recognized by enzymes or processes which can nick the DNA at or near the site of the damage, exchanges of DNA will cause parental DNA containing such lesions to become associated with the new DNA. As a result, nicking the DNA at the lesions will reduce the apparent size of the newly synthesized DNA; such reductions can be detected on alkaline sucrose gradients. Since recombination results in new and old DNA becoming covalently linked, it is not possible to evaluate sizes of new and old DNA using traditional methods. Instead it is necessary to determine by probability modeling the extent of recombination necessary to generate the observed mass distribution. Using these probability models, we are applying them to our own data as well as published data to evaluate the levels of molecular recombination that are present or which could be detected.

MAJOR FINDINGS AND PROPOSED COURSE: We have been able to model recombination events as reassociations of damage between parental and newly synthesized DNA molecules that are either monodisperse or polydisperse in size. It has been possible to determine relationships between frequency of recombination events and number of lesions per molecule required for detection. In this way we can estimate the minimum number of recombination events per experimental condition that can be detected. Applying this method of analysis, we have concluded that small doses of UV suppress over 75% of meiotic molecular exchanges in yeast. These results are supported by genetic evidence. In addition we have concluded that if reciprocal recombination is associated with toleration of UV-induced pyrimidine dimers, the extent of recombination must be less than about 25% per dimer. These results, which are consistent with our analysis of mammalian cell data, stand in marked contrast to those with E. coli. In the latter case, the data is best explained by a reciprocal recombination event being associated with every pyrimidine dimer.

We plan to use this approach to examine the ability of various agents to cause recombination and to determine the timing of recombinational events.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recombination is essential in normal meiosis and in the repair of some types of lesions. In the absence of recombination aneuploidy events are expected in meiotic systems, whereas their absence in mitotic systems may lead to dominant lethality. These studies are part of a general approach to understanding the mechanisms of recombination at the genetic and molecular level in mitotic and meiotic cells and relating them to repair processes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21015-01 CGTB

PERIOD COVERED
December 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DNA Synthesis on Damaged Templates by Yeast Crude Extracts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Moore	I.P.A.	CGTB	NIEHS
	M. Resnick	Research Geneticist	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) We have observed previously that newly synthesized DNA in UV-irradiated mitotic and mieotic yeast cells is substantially larger than the distance between pyrimidine dimers indicating that synthesis can take place past dimers. We have been investigating the ability of pyrimidine dimers to block or allow DNA replication in yeast using the method of Moore and Strauss [Nature 278 (1969) 664] which utilizes in vitro synthesis on primed ØX174 DNA templates that have been UV-irradiated. We have modified this procedure in order to sequence the DNA products synthesized with various polymerases, yeast crude cell lysates, or combinations of these. Synthesis of DNA by the crude extract system or purified yeast DNA polymerase I is terminated by dimers and the extracts do not appear to increase the possibility of read-through by exogenously added purified DNA polymerases (E. coli polymerase I and yeast DNA polymerase I). Using these procedures we are quantitating the extent of termination by DNA damage and comparing the relative synthesis in extracts from mitotic and meiotic cells or cells stressed with UV-irradiation.

METHODS EMPLOYED: Various repair-deficient mutants of Saccharomyces cerevisiae are genetically manipulated and grown using techniques standard for handling yeast. Cell extracts capable of in vitro DNA synthesis are made according to the methods of Sugino and Greenberg [PNAS 78 (1981) 7261]. Templates, primers and sequencing techniques for ØX174 have been described by Moore and Strauss [Nature 278 (1969) 664]. The mutagen treated ØX174 DNA is added to appropriate buffers or extracts and synthesis of defined regions is determined. If DNA damage stops synthesis, the position of stoppage can be determined on sequencing gels.

MAJOR FINDINGS AND PROPOSED COURSE: Using modifications of the Moore and Strauss methods for in vitro synthesis and the Sugino and Greenberg procedure for replication in crude yeast extracts, we have obtained limited DNA synthesis on defined DNA ØX174 sequences. Addition of yeast polymerase to the extract does not greatly increase the amount of synthesis. The irradiation of template DNA with far UV results in the termination of synthesis at sites corresponding to possible pyrimidine dimers. The presence of crude extracts do not appear to lead to generalized read-through.

Using this system we plan to investigate factor(s) that may be involved in "read-through" in intact cells. It is anticipated that the synthesis or activation factors may be induced by irradiation or other DNA damaging agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mutation is considered to be a major source of human disease caused by DNA damaging agents. The mechanisms by which mutations arise is poorly understood in eukaryotic systems. This project will aid in our understanding of the mechanism(s) by which DNA damage is revealed as mutations. Through the use of various repair mutations and defined conditions for synthesizing DNA in crude extracts, it should be possible to characterize some of the early events in mutagenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21016-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Enzymes Involved in DNA Repair and Meiosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terry Chow	Visiting Fellow	CGTB	NIEHS
	M. Resnick	Research Geneticist	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The RAD52 gene in Saccharomyces cerevisiae controls the repair of ionizing radiation induced DNA double-strand breaks, radiation-induced recombination and recombination in meiotic cells. A comparison of crude extracts from logarithmically growing cells of wild-type and rad52 mutants reveals that the mutant lacks an alkaline deoxyribonuclease. When the same alkaline deoxyribonuclease is followed through meiosis, RAD52 extracts exhibit maximum activity at three hours into meiosis with a 50% to 100% increase over the activity at 0 time. No increase is observed for rad52. Extracts from logarithmically growing rho^0 strains, however, also lack this alkaline deoxyribonuclease. But when a more sensitive system is utilized, extracts from rho^0 strains exhibit competition for the antibody while no competition is observed for rad52 extracts. These results are consistent with observations of RAD52 genetic function in rho^0 strains and indicate that the RAD52 controlled alkaline deoxyribonuclease is a processed function in rho^0 strains.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Various repair-deficient mutants of Saccharomyces cerevisiae are grown using standard yeast techniques. To obtain yeast crude extracts, the cells are broken open with a French pressure cell. The screening of alkaline deoxyribonuclease involves the use of a rabbit anti-serum which was raised against a Neurospora crassa SS-DNA-binding endo-exonuclease and is assayed in the presence of 100 mMTris-HCl buffer, pH 8.0 containing 10mM $MgCl_2$. The purification of this alkaline deoxyribonuclease involves the use of standard column chromatography techniques.

MAJOR FINDINGS AND PROPOSED COURSE: In the yeast Saccharomyces cerevisiae, DNA-repair processes are required to protect against external damaging agents and many of the repair mechanisms are involved in mutagenesis. The RAD52 gene is genetically identified as controlling DNA-repair of ionizing radiation damage and DNA recombination. Neither the product of the RAD52 gene nor any other gene involved in DNA repair in yeast has been identified, although in some cases the mechanisms have been inferred.

In screening crude extracts from wild-type, rad52, and other repair mutants, we have found that rho^0 mutants lack an alkaline deoxyribonuclease which is immuno-cross-reactive with the Neurospora anti-serum, but still has immuno-cross-reactive material. The rad52 strains, on the other hand, lack both immuno-cross-reactive material and alkaline deoxyribonuclease. During meiosis in RAD^+ cells this alkaline deoxyribonuclease increases in activity at a time corresponding to early stages of meiotic recombination; no such increase is found in rad52 strains. Thus, it is likely that this alkaline deoxyribonuclease is under the control of the RAD52 gene. These results provide possible routes for analysis of the biochemical events in recombination, and we are currently attempting to purify and characterize this alkaline deoxyribonuclease.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the genetic events under the control of the RAD52 gene in Saccharomyces cerevisiae have been well described, the molecular biochemical events involved are not well understood. The identification of a gene product under RAD52 control, therefore, allows us to identify the relevent biochemical events involved. Furthermore, this work will allow us to understand better the molecular events that surround recombination and DNA repair either in mitotically growing cells or meiotically developing systems. The techniques being developed will enable us to investigate similar mechanisms in geniminal lines of higher organisms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21017-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Metabolic activation by a form of cytochrome P-450 induced by 3,4,5-3',4',5' hexachlorobiphenyl treatment of rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Robertson	Visiting Fellow	CGTB	NIEHS
	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	J. Goldstein	Pharmacologist	STB	NIEHS

COOPERATING UNITS (if any)

Systemic Toxicology Branch, TRTP

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium mutagenicity has been used to examine the metabolic activation of chemical carcinogens by a form of cytochrome P-450 (HCB-448) purified from lives of 3,4,5,-3',4',5' hexachlorobiphenyl-treated rats. This project will identify carcinogens which are substrates for this form of cytochrome P-450, and allow a comparison with other forms purifed from rat liver.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Metabolic activation is being characterized using monooxygenase systems reconstituted from purified components, in the standard Salmonella plate test.

MAJOR FINDINGS AND PROPOSED COURSE: In a reconstituted monooxygenase system, HCB-448 was effective for the mutagenic activation of 2-aminofluorene. In experiments determining time and cytochrome P-450 dependence, HCB-448 gave approximately 2X more mutants than MC-448, the major form in the livers of 3-methylcholanthrene-induced animals.

The ability of HCB-448 to activate compounds from other major classes of chemical carcinogens is in the process of being tested.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Different carcinogens appear to be activated by different forms of cytochrome P-450. Characterization of specific forms is important in understanding possible tissue and species specificity of these chemicals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21018-01 CGTB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of neonatal exposure to Diethylstibestrol and 2,3,7,8-tetrachloro-dibenzo-p-dioxin on the sex differentiation of the hepatic monooxygenase system in the rat.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Robertson	Visiting Fellow	CGTB	NIEHS
	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	G.W. Lucier	Research Chemist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium tester strains have been used to examine the metabolic activation of aflatoxin B_1 by hepatic microsomal preparations from male and female rats exposed to DES and TCDD. This project will determine the ability of these agents, administered during a critical period of neonatal development, to alter the sexual differentiation of the hepatic monooxygenase system.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The hepatic microsomal fraction from control and DES or TCDD treated rats are incorporated in standard Salmonella plate assays using strain TA98 with aflatoxin B_1 (AFB_1).

MAJOR FINDINGS AND PROPOSED COURSE: No difference was observed in the mutagenic activation of AFB_1 by preparations from control male and female rats.

Approximately 2 times more total cytochrome P-450 was required in preparations from DES-treated female rats to give the same number of mutants as with preparations from control animals of both sexes despite a 50% increase in nmole P-450/mg microsomal protein following DES treatment.

No significant differences were found when TCDD-treated male microsomes were compared with controls.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Sex differences have been observed in susceptibility to chemical carcinogens. Monooxygenase systems can be modulated by various treatments. Such studies give information on the possible basis for such sex differences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21019-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Proteins associated with meiosis and DNA Repair

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.A. Resnick	Research Geneticist	CGTB	NIEHS
	M. Volkert	Staff Fellow	LMG	NIEHS
	T. Chow	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

Professor Brian Cox, Oxford University

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: NIEHS	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Unique DNA metabolic activities have been implicated during meiosis and following exposure of mitotic cells to DNA damaging agents. Some of these activities in the yeast Saccharomyces cerevisiae are mediated by common gene products. We are developing methods to enable us to identify meiotic-specific proteins in yeast and relate them to specific repair genes. Proteins from meiotic and mitotic cells that are wild-type or mutated in various repair genes are to be examined on 2-D gels. To increase the sensitivity of the assay and distinguish mitotic from meiotic specific proteins, we have raised antibodies to crude extracts of wild-type cells. Extracts from meiotic cells will be treated with antisera and the resultant proteins will be analyzed. Initial experiments have shown that the activities of several nucleases can be reduced significantly using this method. One of the meiotic genes, RAD52, is being characterized by examining its activity in E. coli. In yeast, the RAD52 gene product is required for DNA double-strand break repair and normal meiotic recombination; the RAD52 gene controls a single-strand deoxyribonuclease. This gene has been cloned into a bacterial plasmid and it may now be possible to characterize the specific RAD52 gene product and determine whether it complements other E. coli repair functions.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard procedures for growing yeast are being used in this study. We are using specially developed strains in which synchronous meiosis can be obtained. Where needed, transformation of yeast or bacteria is according to techniques commonly described in the literature. Various repair deficient mutants such as rad52-1 or rad1-1 (excision repair) are used as needed.

MAJOR FINDINGS AND PROPOSED COURSE: We have previously shown that several mitotically identified repair functions are essential during normal meiosis. In the absence of some of these there is loss of meiotic recombination and cell death. Genetic evidence indicates that some of the corresponding gene functions arise during meiosis rather than being continuously present. We are attempting to identify meiotic-specific proteins and determine those which may be related to DNA repair functions. Crude extracts of repair proficient and deficient strains are being examined at various times during meiosis and following exposure to DNA damaging agents. The proteins are then examined using 2-D gel methods described by O'Farrell. To discriminate between general proteins and meiotic or repair specific proteins, we are raising antibodies to whole cell extracts of wild-type and rad52 strains. Using these antisera we anticipate that it will be possible to decrease the levels of constitutive proteins and thus enrich for meiotic specific proteins.

Using this approach we have been able to examine the RAD52 single-strand deoxyribonuclease. Extracts of wild-type cells taken at various times during meiosis had considerable nuclease activity present. Treatment with antisera raised to extracts of mitotically growing rad52 cells reduced the nuclease activity considerably. The only significant activity remaining was that corresponding to the RAD52 nuclease and this increased over two-fold at the time of recombination commitment in meiosis. Thus, it appears that by using antisera raised against whole cell extracts, it should be possible to eliminate many mitotic type proteins and enrich for those associated with meiosis or induced during DNA repair.

In conjunction with these studies, we are examining the characteristics of the RAD52 gene. The product of this gene is essential for the repair of DNA double-strand breaks induced by ionizing radiation and for recombination during normal meiosis. In rad52 mutants, there is no recombination during the meiotic cycle and cells begin to die with the onset of the meiotic round of DNA synthesis. Thus, the RAD52 gene is dispensable during mitotic growth but becomes essential during meiotic development. As stated above, the nuclease controlled by the RAD52 gene increases during the meiotic cycle. To characterize more fully the RAD52 gene, it has been cloned (by B. Cox) into an E. coli plasmid. Once we are able to recover transformants of E. coli that contain this gene, we will be able to determine more fully the nature of the protein and whether the nuclease we have described above is the product of the RAD52 gene. In addition we plan to examine various repair deficient and recombination deficient strains for complementation by the RAD52 plasmid.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
It is now clear based on genetic studies from several organisms that some mitotically identified repair functions are required for meiosis. In addition there are undoubtedly several meiotic-specific proteins that are involved in the processing of DNA. We are developing an integrated biochemical/genetic approach to understanding DNA metabolic events during the meiotic stage of development. This work with yeast will serve as a model for understanding events in germinal cell lines of higher organisms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21020-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mechanisms of recombination induced by DNA damage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. Resnick Research Geneticist CGTB NIEHS

COOPERATING UNITS (if any)
Professor James Haber, Biology Department, Brandeis University

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: NIEHS	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many types of DNA damage are known to induce recombination. Recent observations with other systems have indicated that there may be a direction to the recombination when nonreciprocal exchanges (gene conversion) occur. We have developed a system in which it is possible to examine whether damage in a chromosome can cause information to be preferentially transferred into or away from the chromosome. Yeast cells of one mating type are treated with the damaging agent and mated with untreated cells. The two cell types have mutations at different sites in the LYS2 gene. Prototrophs are selected and the recessive allele is determined. Using this system, we have found that ionizing radiation-induced gene conversion occurs in over 85% of the cases by the irradiated chromosome being the recipient of information from the undamaged chromosome. These results are consistent with the model proposed by Resnick for the repair of DNA double-strand breaks.

PHS-6040
(Rev. 2-81)

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Recombinational events are induced by DNA damaging agents in nearly all eukaryotic systems examined including human cells in culture. Recombinational mechanisms, may give rise to large reassociations of chromosomes, as for aberrations and/or they may enable the expression of recessive mutations. This work will further our understanding of how DNA damage may induce genetic changes and whether various DNA damaging agents can be categorized according to the type of recombination they cause.

METHODS EMPLOYED: Various strains of Saccharomyces cerevisiae are genetically manipulated and grown using techniques standard for handling yeast. Cells of opposite mating types and carrying mutations at different sites in the LYS2 gene are grown to stationary phase. They are then treated with an agent of interest and mixed to allow mating to occur. The cells are plated to a medium which will only support the growth of the zygotes.

MAJOR FINDINGS AND PROPOSED COURSE: A major portion of this work has been devoted to developing a system in which it is possible to detect the direction in which recombination occurs when recombination is induced by a DNA damaging agent. We have chosen to examine gene conversion with the LYS2 gene. Strains are used which are mutated at two distinguishable sites in this gene (lys2-x) and (lys2-y). When these strains are mated, the resulting zygotes will only grow on lysine deficient medium if there has been a recombinational event. The great majority of events involve gene conversion. For example, lys2-x goes to "+" or vice versa with the "+" information being provided by the corresponding region in the homologous chromosome. To determine the nature of the gene conversion events, the prototrophs are examined for the presence of a recessive allele by plating the zygote colony to medium containing alpha-aminoadipic acid. Since this medium selects for lys2 mutants, it is possible to select for the recessive mutation (lys2-x/+ goes to lys2-x/lys2-x). Because the two alleles are distinguishable (i.e., one is temperature-sensitive and the other is UV-revertible) it is possible to evaluate the nature of the recessive allele in the original prototroph and, thus, the direction of information transfer.

We have used this system to examine ionizing radiation induced recombination. As previously shown, the repair of DNA double-strand breaks involves recombination and, as had been theorized, it appeared that an initial stage involved gene conversion type intermediates. The LYS2 system enabled us to examine the nature of the recombinational events. Irradiated cells of one mating type and lys2-x were mated with unirradiated lys2-y cells of opposite mating type. Among the resulting recombinants, over 85% arose by a gene conversion event which involved transfer of information from the undamaged to the damaged chromosome. In other words, the broken chromosome was the recipient of information. In the absence of such recombination, other evidence would suggest that the damage would be lethal.

This system will also enable us to examine the nature of recombination events outside the LYS2 gene that are associated with the initial selected event at this gene. Using this system we plan to examine various DNA damaging agents to determine whether there are classes of agents that yield different patterns of direction in recombination. It is possible that single-strand damaging agents cause gene conversion in both directions while double-strand agents (such as ionizing radiation and bleomycin) result in preferred directions of recombination.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21028-01 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies on the Salmonella plate test

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	D. Pagano	Research Microbiologist	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of separate projects are currently being carried out, the results of which will have effects upon different aspects of the Salmonella test system. One project is involved in looking at the storage life of mutagens used as control compounds. The mutagenicity of freshly prepared solutions is periodically checked against previously prepared solutions stored at -20°C and -80°C.

Another project is studying the growth of the Salmonella tester strains on minimal media. The results of this study will be used to assess the effect of growth rates on the production of mutations and also what the effects are on mutagens of different half-lives.

A third project is the suppression of mutagenic responses by substances which affect the Salmonella tester strains. When tested along with known mutagens, these inhibitors depress the appearance of his+ colonies in a dose-dependent manner at doses which produce no apparent toxic effects, as determined by visual examinations of the background lawn on the plate.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames' Salmonella/microsome spot test or plate incorporation tests are being used with slight modifications. Viable counts are made by taking plugs from the lawn, diluting and plating at various times.

MAJOR FINDINGS AND PROPOSED COURSE: Five freshly prepared mutagens, 3 direct-acting, i.e., Sodium Azide (NaN_3), 4-nitro-o-phenylene diamine (4NOP) and 4-nitro-quinoline oxide (4NQO) and 2 mutagens which require S9 activation, Benzo(a)pyrene (BAP) and 2-aminoanthracene (2AA), freshly prepared, are being compared with preparations made in advance and stored at -20°C and -80°C. To date testing has been completed on samples stored for 108 days. Preliminary results suggest that 4NOP, 4NQO and perhaps BAP have remained stable. 2AA has begun to show a decline in mutagenic activity with storage while NaN_3 appears to increase in activity with storage. Testing is still being done periodically.

Growth curves on minimal media were established for strains TA98 and TA100. There was approximately a 3-4 hour lag time with growth abating between 8-12 hours. Varying the levels of histidine/plate gave proportional levels of growth, i.e., 0.5X histidine level (compared to normal 1X) grew as if there were 1/2 the number of cells under normal conditions, .2X histidine grew 1/5 as much, etc., but varying both the number of cells plated and the levels of histidine, did not produce the expected patterns of growth. Further work is being done on the growth conditions before studies using mutagens will be started.

Approximately 25 compounds including antibiotics, pesticides, fatty acids, anti-oxidants, dyes and solvents have been screened by the disc method against 6 mutagens, both direct-acting and requiring S9 activation for the production of a zone of inhibition of his^+ colonies. Microscopic examination (40X) of the background lawns in this area suggests that no toxicity is present, but examination under 400X magnification reveals toxic effects, i.e., elongated or filamentous cells and decreased micro-colony density.

Expanded studies with ampicillin, crystal violet, caprylic acid, 2-mercapto-ethanol, 2-phenylethanol and lauric acid have produced various degrees of inhibition. The inhibition depends upon the type of inhibitor as well as the mutagen used. Studies are continuing in order to further describe this phenomenon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Salmonella (Ames') test is currently the most widely used bacterial mutation assay and has an extensive data base of results to draw upon. The relationship between mutagenesis and carcinogenesis appears to depend highly on the class of chemical under consideration and on an understanding of the test systems. The work being conducted here will provide a better understanding of the Ames' test, resulting in a greater degree of accuracy when interpreting results and predicting the effects in other systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60101-04 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of a Computerized Data Base Management System for the Cellular and Genetic Toxicology Branch

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Other:	M. Rowley	Computer Systems Analyst	BRAP	NIEHS

COOPERATING UNITS (if any)

Biometry and Risk Assessment Program
Bolt, Berenak & Newman, Inc., Cambridge, Mass.

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The NTP Mutagenesis Testing Programs are generating large volumes of data and experimental information on all chemicals which will be tested for mutagenicity. A computerized data base management system is needed to capture this information in an interactive mode in the testing laboratory, store, process, and analyze the data, and provide summary analyses to the experimenter and to the CGTB project officers. This system will also allow the CGTB staff to follow the course of testing with time in the large number of laboratories regardless of the mutagenicity test system being used. The PROPHET system developed and managed under contract to NIH/DRR, has been selected. It is being adapted to serve as the CGTB data base management system.

PROJECT DESCRIPTION

METHODS EMPLOYED: An ad hoc advisory group was formed to aid the NIEHS personnel in defining computer needs and the identifying of an acceptable system.

MAJOR FINDINGS AND PROPOSED COURSE: A system survey identified one system, PROPHET, developed for NIH DRR by Bolt, Beranek and Newman (BBN) which would meet the needs of the NIEHS.

Work has been completed at BBN on a laboratory data entry terminal to be used for data collection and on the data base structure and software required to store this data on PROPHET. The data entry terminals are in place in the Salmonella testing laboratories and are being used to transmit data directly to PROPHET thereby obviating the need for data forms. Once in PROPHET the data is checked for completeness and made available to the CGTB project officer in an easily readable form for approval. After approval, the data is stored in readily accessable tables for future reference and summary management tables are updated with the new information. Data for other test systems are being entered into PROPHET by NIEHS personnel. Drosophila and cytogenetics data are being entered into the system at NIEHS.

Analytical techniques required to perform mutagenicity and quality control determinations are being developed by NTP scientists for addition by BBN into PROPHET. Finally, BBN had developed additional interactive and batch report generation routines to facilitate the management of the mutagenicity results.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This system will allow the timely and accurate collection and retrieval of laboratory test data, and will also provide NTP management with a tool to manage the results, produce management reports and provide analyses to support mutagenicity determinations. It is the first system of its type to provide all of these facilities in such a fashion for use by non-computer-oriented management personnel.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60102-04 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Testing of Chemicals of Interest in Salmonella

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	D. Pagano	Research Microbiologist	CGTB	NIEHS

COOPERATING UNITS (if any)
North Carolina Central University, Biology Department
LEB, NIEHS
Department of Toxicology, University of Louvain, Belgium

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium strains TA97, TA98, TA100, TA1535, TA1537 and TA1538 are being used to test chemicals of interest for mutagenicity. Dimethylaminoazobenzene (Butter yellow, DAB) and three of its structural analogues, N-methyl-5-phenylazo-indoline (IND), 4-N-pyrrolidinylazobenzene (PYR), and 4'-diethyl-4-N-pyrrolidinylazobenzene (EPYR); 2,3,7,8-Tetrachlorodibenzofuran (TCDF); two alkylnitrites; 4-chloromethylbiphenyl (4CMB); a lactone and its ester precursors; and 9-aminoacridine (9AA) and quinacrine were tested or will be tested for their mutagenic activity using various in vitro activation systems.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test procedure of Ames with some modification or liquid suspension test were used.

MAJOR FINDINGS AND PROPOSED COURSE: Butter yellow and all of its analogues were mutagenic and required 30% S9 for the observed activity. When comparisons of the in vitro mutagenic responses are made on the initial responses, EPYR produces a greater mutagenic response than PYR and DAB with IND being weakly mutagenic. In transformation studies with BHK-21 cells, both PYR and IND were roughly equivalent to each other and more active than DAB (EPYR was not tested). In carcinogenesis studies, DAB was a more effective carcinogen than EPYR; IND and PYR did not produce tumors in rats in a limited study. It is clear that the relative activities of the chemicals in the Salmonella mutation assay, BHK-21 transformation assay, and rat carcinogenesis assay are not the same, and that the carcinogenicity of this class of chemicals, at least in the limited tests reported, cannot be predicted from the observed mutagenicity and cell-transforming ability.

TCDF was tested with and without Aroclor 1254-induced rat liver S9 at up to 100 µg/tube in a pre-incubation assay. No mutagenicity or toxicity was observed with any of the strains used.

Two alkylnitrites produced very similar patterns of mutagenicity and toxicity. Without S9 activation, both compounds were non-mutagenic to strain TA98 but showed a slight, dose-related increase in revertants when tested in TA100 (maximum response at 3000 µg/plate). In the presence of S9, both compounds exhibited slight, dose-related increases in mutagenicity (maximum response at 3000 µg/µl for TA98, 4000 µg/µl for TA100). The toxicity of each compound was similar with a higher concentraion being toxic in the presence of S9 (6000 µg/plate with S9 vs. 4000 µg/plate without S9).

4CMB was tested in Salmonella strains TA1535, TA1537, TA98 and TA100. Reproducible dose-related positive responses were obtained in strains TA1537, TA98 and TA100 in the presence and absence of S9, the response being greater in the absence of S9. With TA1535, dose-related increases in revertants/plate were observed only in the absence of S9. Slight toxicity was noted and occurred at higher doses with S9 (200 µg/plate) than without (50-100 µg/plate).

A complex lactone and 4 ester compounds which may be precursors for the lactone were tested with and without S9 in strains TA100, TA98, and TA1537. All compounds were non-mutagenic but produced toxic responses at doses above 500 µg/plate both with and without S9.

9-Aminoacridine and another aromatic amine, quinacrine are currently being tested using the standard S9 preparation and a horseradish peroxidase activation system. This is to determine the effects of different activation systems on the mutagenicity of those substances which are mutagenic in the absence of exogenous activation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
TCDF and the alkylnitrites are substances to which the population is exposed. Examination of their mutagenicity will provide information that can be used by individuals or organizations interested in these substances.

The results with DAB and its analogues have provided structure-activity information which may be useful in predicting the toxicity of other, related substances. Additionally, the mutagenicity results have been shown to have no correlation with transformation or carcinogenic endpoints, showing that for this class of compounds, mutagenicity in bacteria may not be a good indicator of other genotoxic effects.

4CMB was tested as part of the Genetic Toxicology Trial sponsored by the U.K. Environmental Mutagen Society. The positive results obtained in our study have been compared to other bacterial test results as well as the results in other, higher organisms with many endpoints. These comparisons will be useful in validating systems as well as aiding in the development of multi-test system schemes.

The lactone and ester compounds have been synthesized as potential anti-tumor agents. Therefore, the negative mutagenicity results are significant in light of the fact that many anti-tumor agents are mutagenic in this test system.

The results that will be obtained with 9-aminoacridine and quinacrine will be compared with the results from DNA binding studies and may provide useful information about the metabolites formed by the different activation systems and the reactive product that ultimately interacts with DNA.

PUBLICATIONS

Zeiger, E. and J. Guthrie (1981). Cyclic hydrazides are mutagenic for Salmonella typhimurium. Mutation Res. 91: 199-205.

Zeiger, E. and D.A. Pagano (1982). Comparative Mutagenicity of Dimethylaminoazobenzene and Analogues in Salmonella. Carcinogenesis. 5: in press.

Pagano, D. and M.D. Shelby. (1982). Mutagenicity of 4-chloromethylbiphenyl in the Salmonella/microsome assay. Mutation Res. 100: 67-70.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 60105-04 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Genetic Control of Mutation in Drosophila

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.M. Mason Geneticist CGTB NIEHS

COOPERATING UNITS (if any)
B. Slatko, Department of Biology, Williams College, Williamstown, MA
University of Leiden, The Netherlands

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) It has become evident in the last several years that mutation rate is under genetic control. In bacteria and yeast the frequency of induced mutations can be either increased or decreased by blocking one or another pathway of DNA repair. This project is designed to determine the relationship between DNA repair and mutagenesis in Drosophila melanogaster. Two approaches are being taken: (1) A mutant which increases the mutation frequency (a mutator) has been identified, mapped, and characterized; and (2) the interaction of DNA repair defective mutants and hybrid dysgenesis (naturally occurring mutators) has been observed in double mutant combinations.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized mutants and chromosomal aberrations in Drosophila melanogaster are employed.

MAJOR FINDINGS AND PROPOSED COURSE: One mutator being examined is unable to repair X-ray induced breaks in the normal way. In its presence broken chromosomes are recovered which appear to be deficient for a telomere (on both cytological and genetic grounds). That is, unlike X-ray induced aberrations in wild-type, the broken chromosomes do not appear to be capped by any previously existing telomere. The mutator is recessive and maps near the end of the left arm of chromsome III. It is active throughout oocyte development, but does not appear to be active during spermiogenesis. The mutator does not increase the frequency of meiotic nondisjunction. It does, however, increase the frequency of X-chromosome loss. These losses are the results of whole arm deletions in which the centromere is recovered but the rest of the X has been lost. Cytological studies of some of these are being made.

Certain naturally occurring mutators ("hybrid dysgenic strains") have very specific phenotypes in terms of where in the genome they allow mutation, chromosome breakage or recombination to occur. It has been hypothesized that the genetic changes induced in the presence of hybrid dysgenesis are the result of DNA sequences inserting into or excising from genomic DNA. If this is the case the cell's DNA repair capacity may play a role in movement of these sequences. Therefore, combinations of DNA repair deficient mutants and hybrid dysgenic factors have been made to allow the study of quantitative changes in mutation, recombination and segregation. In such combinations the recovery of 3 independent dysgenic factors from a hybrid male are seen to decrease from about 0.4% in the controls to almost zero when in the presence of the repair deficient mutants mei-41^{D1} or ^{D5}.

Studies are also underway at the University of Leiden to compare the effects of neutrons and high LET particles with X-rays and chemicals for the induction of chromosome damage, mutation, and telomere loss in the mutants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will lead to an understanding of the cellular mechanisms used to regulate the rates of mutation and chromosome breakage in eukaryotic organisms. It should in the long run, allow one to sequence a telomere and thereby obtain additional information about the organization of the eukaryotic chromosome.

PUBLICATIONS

Mason, J.M.: (1980) Spontaneous mutation frequencies in mutagen-sensitive mutants of Drosophila melanogaster. Mutation Res. 72: 323-326.

Mason, J.M., M.M. Green, K.E.S. Shaw and J.B. Boyd (1981). Genetic analysis of X-linked mutagen-sensitive mutants of Drosophila melanogaster. Mutation Res. 81: 329-343.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60106-04 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cytogenetics Analysis of Mutagen-Sensitive Mutants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Mason	Geneticist	CGTB	NIEHS
	N.N. Scobie	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

J.B. Boyd, Department of Genetics, University of California, Davis, CA

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mutagen-sensitive mutants defective in DNA repair mechanisms are collected in Drosophila melanogaster. The mutants are characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. The tests used in the initial characterization of these mutants include genetic and cytogenetic mapping, complementation analysis, tests for sensitivity to unrelated mutagens, and tests for pleiotropic effects on related functions such as recombination. A fine structure map of the mei-41 region has been constructed to ascertain the allelism relationship between mus104, and mei-41, and to confirm the large size of mei-41 found during mutational analysis. Many mei-41 alleles are temperature sensitive. A sample of temperature sensitive alleles already mapped is seen to be distributed throughout the locus.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized X-linked mutants and chromosomal aberrations in Drosophila melanogaster are employed. Because the mutagen-sensitive (mus) mutants are X-linked the presence of these mutants is monitored by mating mus males to attached-X females, treating the progeny with MMS (or other mutagen), and checking the sex ratio of the survivors.

MAJOR FINDINGS AND PROPOSED COURSE: A fine structure map of a portion of the X Chromosome has been constructed to clarify the allelic relationships between mutants at two putative mus loci, mus(1)104 and mei-41.

The results so far lead to the following conclusions. (a) Two mus 104 alleles map within the mei-41 locus and thus are allelic. (b) If mus 104 and mei-41 are allelic they cannot define two different genes or two different pathways of post-replication repair as proposed by Boyd et. al. (c) Since mei-41 and mus 104 have different effects on meiosis but the same effect on sensitivity to mutagens it is possible to uncouple the effects of mutants of this locus in different tissues. The reason for this uncoupling may become evident after other alleles are added to the map of this region. (d) The mei-41 locus is very large in recombinational terms (0.25 centimorgans). It is the largest locus known in Drosophila and 50 X the size of a simple gene such as ry. This is consistent with the observation that mei-41 is about 25 X the size of a typical mus X-linked gene in a mutational study. Making the usual assumptions as to the genome size and the amount of recombination in Drosophila we calculate that the mei-41 locus contains about 145 kb and that if a protein product is made it should be about 5 X 10^6 daltons. Mei-41 alleles are being tested for temperature sensitivity, based upon female sterility. Of approximately forty alleles tested, 78% demonstrate temperature sensitivity. Temperature sensitive alleles already mapped are found spread through the locus. The mapping of more t.s. alleles will provide, in conjunction with additional complementation data, information about the structural organization of this very large "complex" locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the action of genes controlling mutagen sensitivity is necessary for understanding DNA repair, mutagenesis, recombination and chromosome stability.

PUBLICATIONS

Mason, J.M., Green, M.M., Shaw, D.E.S., and Boyd, J.B.: Genetic analysis of X-linked mutagen-sensitive mutants of Drosophila melanogaster. Mutation Res. 81: 329-343 (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60114-03 CGTB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Activation of Polycyclic Hydrocarbons and other compounds to Mutagenic products by Prostaglandin Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Robertson	Visiting Fellow	CGTB	NIEHS
	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	T. Eling	Head, Prostaglandin Group	LPFT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Function and Toxicology, NIEHS

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prostaglandin synthetase is capable of metabolizing a number of substances previously thought to be metabolized only by the mixed-function oxidase system. A method has been developed for testing the ability of prostaglandin synthetase to metabolise xenobiotics to products mutagenic to Salmonella.

Benzo(a)pyrene, benzanthracene, chrysene and a number of their metabolites have been tested for mutagenicity after activation by prostaglandin synthetase.

A number of aromatic amines and related compounds including the bladder carcinogen benzidine, nitrosamines, and the phthalate derivative DEHP, have also been tested.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test has been modified to circumvent the toxicity of the detergent used to solubilize the prostaglandin synthetase and of the arachidonic acid used as a cofactor in the reaction. A crude microsomal preparation containing prostaglandin synthetase is prepared from ram seminal vesicles. This preparation is sterilized by filtration and included in a pre-incubation modification of the standard Salmonella plate test.

MAJOR FINDINGS: In the presence of the prostaglandin synthetase substrate, arachidonic acid, only the dihydrodiols of benzo(a)pyrene, benzanthracene, and chrysene, which allow formation of the bay-region diol epoxide, were metabolized to mutagenic products by ram seminal vesicle microsomes. This activity was inhibited by the prostaglandin synthetase inhibitor indomethacin, and was comparable to that achieved with a Aroclor-1254 induced rat liver S-9 cytochrome P-450 system. Cytochrome P-450, unlike the prostaglandin synthetase system, was also able to activate the parent compounds and several other dihydrodiol derivatives. Thus, the prostaglandin synthetase system appears more selective than the cytochrome P-450 system in the conversion of polycyclic hydrocarbons and their metabolites to mutagenic products.

Of the aromatic amines tested, benzidine, 2-aminofluorene, 2-naphthylamine, and 2,5-diaminoanisole were metabolized to mutagenic products by the prostaglandin synthetase system. 1-Naphthylamine, 2-aminoanthracene, 2-acetylaminofluorene, and 2,4-diaminoanisole were negative or weakly mutagenic. The other compounds tested: N-nitrosodimethylamine, N-nitrosomorpholine, the pesticide Aminocarb, and the phthalate derivative di(2-ethylhexyl)phthalate, were not mutagenic.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most current research on the activation of xenobiotics is with cytochrome P-450 systems. Prostaglandin synthetase is ubiquitous throughout mammalian tissues and frequently co-exists with cytochrome P-450. There is increasing evidence that prostaglandin synthetase may serve as an alternative or complementary activation system in vivo. This work will help to further elucidate the role of prostaglandin synthetase in the metabolic activation of xenobiotics.

PUBLICATIONS

Guthrie, J., I.G.C. Robertson, E. Zeiger, J. Boyd, and T. E. Eling (1982). Activation of several polycyclic aromatic hydrocarbons to mutagenic products by prostaglandin synthetase. Cancer Res. 42, 1620-1623.

Robertson, I.G.C., K. Sivarajah, T. Eling, and E. Zeiger. Activation of some aromatic amines to mutagenic products by prostaglandin synthetase. Cancer Res. (to be submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60116-03 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Development of a Disc Method for the Rapid Identification of Ames Tester Strains

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	D. Pagano	Research Microbiologist	CGTB	NIEHS
	I. Robertson	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A simple scheme has been developed for confirming the phenotype of the standard set of Salmonella typhimurium tester strains. This scheme employs a series of filter paper discs impregnated with diagnostic mutagens or bacterial toxins. Up to 6 diagnostic discs can be placed on a petri dish to test a single Salmonella strain. The Salmonella are distinguished by their responses to ampicillin, crystal violet, nitrofuratoin, 9-aminoacridine, 4-nitro-o-phenylenediamine and sodium azide. The discs have maintained their activity in storage for two years.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella spot test procedure of Ames is used.

MAJOR FINDINGS AND PROPOSED COURSE: It is possible, using various combinations of the discs with each strain, to identify the strain and to confirm its genotype. Strains TA-98 and TA-100 which contain the plasmid pKM101 are resistant to the toxic effects of ampicillin; all strains should contain a deep rough (rfa) mutation and are, therefore, all sensitive to the killing effects of crystal violet. Only TA-100 should be mutagenized by nitrofurantoin; sodium azide will mutagenize both TA-100 and TA-1535. 9-Aminoacridine is specific for TA-1537, however, 4-nitro-o-phenylenediamine will mutagenize both TA-98 and TA-100. This method is rapid and easy to use and provides identification and confirmation of the tester strains at the time of their use in mutagenicity testing. Impregnated discs have remained stable under the proper storage conditions longer than 24 months. Additionally, strain TA97, which has recently been constructed by Dr. Bruce Ames' lab., was tested for its reactivity with the standard discs. Since it contains the plasmid pKM101, it is resistant to the toxic effects of ampicillin; sensitive to crystal violet; positive to both 4-nitro-o-phenylenediamine and 9-amino-acridine but negative to nitrofurantoin. This strain can, therefore, be distinguished from the other strains by its unique pattern of reactivity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Ames/Salmonella test enjoys widespread use in laboratories of all types and sizes. Controls (positive mutagens) should be run at all times. In addition the strain's genotypes should be checked at all times. The method developed here eliminates excess handling of mutagens (carcinogens) for control plate tests, provides a fast and inexpensive positive control system and allows for the positive identification of each strain used at the time of its use.

PUBLICATIONS

Zeiger, E., D. Pagano and I.G.C. Robertson (1981). A Rapid and Simple Scheme for confirmation of Salmonella Tester Strain Phenotype. Environ. Mutagen. 3: 205-209.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 60120-03 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Metabolic Activation of Known Carcinogens by Rabbit Lung to Products Mutagenic to Salmonella

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Robertson	Visiting Fellow	CGTB	NIEHS
	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	R.M. Philpot	Research Chemist	LP	NIEHS

COOPERATING UNITS (if any)
Laboratory of Pharmacology

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium tester strains have been used to examine the metabolic activation of chemical carcinogens by rabbit liver and lung cytochromes using mutation as the endpoint. This project is designed to study the ability of different forms of cytochrome P450 present in lung tissue to activate known carcinogens to mutagenic metabolites. It will also allow a comparison to be made on the relative metabolic activating abilities of lung and liver.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal fractions from rabbit lung and liver are incorporated in standard Salmonella plate and/or pre-incubation tests. Metabolic activation is further characterized using monooxygenase systems reconstituted from purified components in the Salmonella test. Such characterization includes the effects of antibodies to, and inhibitors of, specific forms of cytochrome P450.

MAJOR FINDINGS AND PROPOSED COURSE: In reconstituted monooxygenase systems containing the major pulmonary cytochrome P-450 isozymes ($P\text{-}450_I$ or $P\text{-}450_{II}$), $P\text{-}450_{II}$ was highly effective in the activation of 2-aminoanthracene (2AA), 2-aminofluorene (2AF) and 2-acetylaminofluorene (2AAF), whereas these substrates were not activated by $P\text{-}450_I$. This difference was confirmed by the results of antibody inhibition studies carried out with pulmonary microsomal preparations. The higher activity of pulmonary relative to hepatic preparations was accounted for by the relatively high proportion of $P\text{-}450_{II}$ in the lung (approx. 50%) However, antibody to $P\text{-}450_{II}$ did inhibit the hepatic microsomal activity by 50-70% indicating that $P\text{-}450_{II}$ is important in the activation of these agents in both tissues even though it is a minor component (less than 5% in the liver.

Treatment of rabbits with phenobarbital but not ß-naphthoflavone increases the rate of activation of 2AA and 2AF by hepatic microsomal preparations. This increase is a function of an increase in P-450 form 5 ($P\text{-}450_{II}$). Phenobarbital also induces form 2, as measured by p-nitroanisole N-demethylation. This is the first demonstration of the induction of phenobarbital of two activities catalyzed by different forms of cytochrome P-450.

A significant difference relative in the mutagenic activation of 2AA and 2AAF in pulmonary and hepatic preparations has been observed. The basis of this difference has been examined using deacetylase inhibitors and HPLC analysis of AAF metabolites. This difference can be accounted for by the lower deacetylase activity in the pulmonary compared to the hepatic fraction.

However, no N-hydroxy-AAF is produced from AAF by pulmonary microsomes. The major metabolite is AF and it appears that the pathway for pulmonary microsomal activation of 2AAF is by deacetylation to form N-hydroxy-2AF. N-hydroxy-2AAF is detected in incubations containing hepatic mircosomes, but the major metabolite again is 2AF, and it appears that at least 50% of the hepatic activation of 2AAF also occurs through 2AF. These results indicate the possible importance of 2AF in the mutagenic activation of 2AAF. This contrasts with the accepted view of N-hydroxy-AAF formation being the first step in this process. The metabolism of the aromatic amines to mutagenic products is, to date, the only activity identified for $P\text{-}450_{II}$, but not $P\text{-}450_I$, in the rabbit lung. This activity is therefore being used as a marker for $P\text{-}450_{II}$ in experiments on the induction and repression of cytochrome P-450 by Aroclor 1254 and steroids.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
The susceptibility of the lungs to many chemical carcinogens that are not carcinogens in the liver raises intriguing questions as to basis of difference. Both organs contain P450-dependent monooxygenase systems which have been implicated in the metabolic activation of carcinogens.

PUBLICATIONS

Robertson, I.G.C., R.M. Philpot, E. Zeiger and C.R. Wolf (1981). Specificity of Rabbit Pulmonary Cytochrome P-450 Isozymes in the Activation of Several Aromatic Amines and Aflatoxin B_1. Molecular Pharmacology. 20: 662-668.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60122-03 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of DNA Repair in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Resnick	Research Geneticist	CGTB	NIEHS
	S. Stasiewicz	Biological Laboratory Technician	CGTB	NIEHS

COOPERATING UNITS (if any)
R. Reynolds, Harvard School of Public Health, Radiobiology Division
J.C. Game, University of California, Berkeley, Department of Genetics

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: NIEHS	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) DNA repair mechanisms which have been identified in mitotically growing cells of the yeast Saccharomyces cerevisiae are being examined for their ability to protect cells undergoing meiosis from DNA-damaging agents. We have developed sucrose gradient techniques to examine repair in mitotic and meiotic cells after low doses of UV and ionizing radiation to wild-type and repair-defective strains. Using these techniques we are determining the role of mitotically identified repair functions on damage occurring during meiosis and correlating them with genetic events. There appears to be only one system for excision-repair throughout meiosis and that is controlled by the radl gene product. Cells can tolerate approximately 1500 pyrimidine dimers per cell during the early stages of meiosis due to an ability to synthesize DNA past dimers; as cells proceed through meiosis the damage has a greater lethal effect. These results are explained by bypass synthesis that is not associated with molecular recombination; on the contrary, the damage appears to depress recombination at the molecular and genetic levels. The observed loss in survival is probably due to effects on chromosomal disjunction resulting from loss in recombination.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various repair-deficient mutants of Saccharomyces cerevisiae are genetically manipulated and grown using techniques standard for handling yeast. All of the strains have been developed genetically to exhibit a high level of synchronous meiosis. For studies involving the repair of DNA, cells are irradiated or treated at various times during meiosis with UV or other mutagens; they are then tested for mutation induction and survival. To examine molecular events, the radioactively-labeled cells are examined for the appearance of breaks using sucrose gradient techniques and for the repair of UV-induced pyrimidine dimers. The presence of UV-induced pyrimidine dimers is indicated by sensitivity of the DNA to the endonuclease activity in M. luteus extract which is specific for pyrimidine dimers. In studying the role of repair mechanisms during meiosis, wild-type and repair-deficient strains are tested at various times for recombination and plating efficiency as well as the appearance of DNA strand breaks.

MAJOR FINDINGS AND PROPOSED COURSE: Meiosis is a fundamental developmental stage which occurs in nearly all eukaryotes. Although there is considerable information on the genetic and morphological changes that take place, relatively little is known about DNA metabolic events, DNA repair, or mechanisms of mutation. Since DNA metabolism and recombination in meiotic cells are different from those processes in mitotic cells, the repair capabilities might be expected to differ considerably from those in mitotically growing cells. For example, there may be repair mechanisms that are unique to meiosis or there may be unique levels of repair capabilities. This research represents an integrated attempt to examine DNA repair mechanisms in a well-characterized mitotic/meiotic system at both the molecular and genetic level.

We have developed a lysis and sucrose gradient technique which enables the identification of full-size chromosomal DNA in yeast. A recently improved method, which involves a gentle lysis of cells and is not affected by the post-irradiation or meiotic fragility of cells, allows for the detection of a small number of breaks or pyrimidine dimers per chromosome. With this method we have been able to examine excision repair and post-replication repair after low doses of UV (2-4 J/m^2) to mitotic and meiotic cells and correlate the molecular observations with genetic results. The only excision repair mechanism that exists in cells undergoing meiosis is that controlled by the RAD1 pathway. In the absence of this pathway cells are extremely sensitive to UV throughout meiosis and the spore (haploid) products exhibit a factor of four increase in sensitivity over mitotically growing haploids. However, meiotic cells are able to tolerate several hundred pyrimidine dimers due to an ability to synthesize past dimers (as was previously shown for mitotic cells). The bypass synthesis is not associated with molecular recombination. These observations correlate well with genetic results. DNA damage at the beginning of meiosis decreases the meiotic levels of gene conversion and nearly abolishes reciprocal recombination. The observation that DNA damage induced early in meiosis can cause lethality later in meiosis can now be understood as being due to the abolishing of normal recombination and which in turn would result in nondisjunction of chromosomes during meiosis I.

Using these techniques we are investigating the repair of damage due to other types of agents, particularly low levels of ionizing radiation and various mutagenic agents, during mitotic growth and meiosis. We are also utilizing low levels of DNA damage for measuring normally occurring reconbinational events.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
DNA repair mechanisms are of fundamental importance in the process of mutageneis and ultimately in carcinogenesis. Using yeast as a model lower eukaryote, we have been able to dissect and analyze at least two pathways of DNA repair at the molecular level in growing and meiotic cells. Since these pathways are involved in mutagenesis, this work will further our understanding of the basic mechanism of mutation. In addition this work enables a genetic and molecular examination of the importance of DNA damage in mitotic and meiotic systems and the relevance of DNA repair in these two stages of development.

PUBLICATIONS

Resnick, M.A., J. Boyce, B. Cox. Postreplication repair in Saccharomyces cerecisiae (1981). J. Bacteriol. 146: 286-290.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60123-03 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DNA Repair Processes During Meiosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Resnick	Research Geneticist	CGTB	NIEHS
	T. Chow	Visiting Fellow	CGTB	NIEHS
	S. Stasiewicz	Biological Laboratory Technician	CGTB	NIEHS
	J. Nitis	Guest Worker	CGTB	NIEHS

COOPERATING UNITS (if any)

Dr. Robert Roth, Department of Biology, Illinois Institute of Technology, Chicago, Ill. and Dr. John Game, Dept. of Genetics, U. of California, Berkeley, CA

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: NIEHS	PROFESSIONAL:	OTHER:
1.9	0.9	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using the yeast Saccharomyces cerevisiae we are examining mitotically identified DNA repair systems during normal meiosis. Several mutants in the UV and X-ray repair pathways are being used: rad1 (excision), rad52 (X-ray) and rad6 (mutational). The initiation of meiotic DNA synthesis is normal in the mutants and excision repair mutants are like wild-type for the other aspects of meiosis: recombination, haploidization, and size of DNA. In rad6, rad52 and rad50 mutants, recombination is abolished and for rad52 and rad50 the spore products are inviable. The rad52 mutant accumulates single-strand interruptions (SSI's) with the onset of premeiotic DNA synthesis and their occurrence coincides with commitment to cell death. Based on an absence of SSI's in a rad50 mutant and corresponding genetic observations, the RAD50 gene product precedes RAD52 in the molecular processing of DNA during meiosis. The RAD50 gene product may enable breaks or gaps to occur and the RAD52 gene product, which controls a single-strand deoxyribonuclease may process these gaps. In the absence of correct processing, the interrupted meiotic events lead to cell death.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various repair-deficient mutants of Saccharomyces cerevisiae are genetically manipulated and grown using techniques standard for handling yeast. All of the strains have been developed genetically to exhibit a high level of synchronous meiosis. To examine molecular events, the radioactively-labeled cells are examined for the appearance of breaks using sucrose gradient techniques. In studying the role of repair mechanisms during normal meiosis, wild-type and repair-deficient strains are tested at various times for recombination and plating efficiency as well as the appearance of DNA strand breaks.

MAJOR FINDINGS AND PROPOSED COURSE: In the yeast Saccharomyces cerevisiae DNA repair processes are required in mitotically growing cells to protect against external damaging agents, and most of the repair mechanisms are involved in mutagenesis. We are investigating the role of various repair systems during the meiotic stage of development in terms of their importance to normal meiosis. Mutations in the RAD6 gene, which is required for UV-induced mutagenesis, do not prevent meiotic DNA synteheis; however, meiotic recombination and meiotic products are not observed. Mutations in the RAD52 pathway also enable the meiotic round of DNA synthesis and meiotic spore products are produced; in this case the spores are inviable and again no recombination is detected. Mutations in a third pathway of DNA repair, excision repair, do not appear to affect meiosis. Mutants of the RAD1 gene exhibit normal DNA synthesis, recombination and sporulation and the chromosomal DNA does not have interruptions during meiosis.

It was established previously that rad52 mutants lack the ability to undergo radiation-induced mitotic recombination and for the case of X-rays there is an absence of double-strand break repair. We concluded that double-strand break repair involved recombinational mechanisms. Reasoning from this and the genetic effects of rad52 on meiosis, we began to examine the chromosomal DNA of wild-type and rad52 strains throughout meiosis. The wild-type exhibits no changes in single- or double-strand size, indicating that if recombination associated breaks occur during meiosis, they are short-lived. Unlike the wild-type, the rad52 mutants accumulate single-strand interruptions (SSI's), during meiosis. Their appearance requires the initiation of DNA synthesis and they are found in newly synthesized and parental strands. Double-strand breaks are not observed; however, we have not precluded them as a short-lived intermediates in the RAD^+ strains. The number of breaks, which is about 200 per cell, correlates well with the genetic exchanges in meiotic cells. From these results we have concluded the the RAD52 gene product is involved in the early stages of recombination during meiosis (as well as during mitotic growth) and in the rad52 mutants the cells are blocked at a stage which results in SSI's. The lethality in the rad52 mutant during meiosis corresponds to the appearance of the DNA interruptions and indicates that unresolved recombination structures may cause lethality.

We have also examined a rad50 mutant which, based on genetic evidence, is blocked at an earlier step in meiosis. Since no SSI's are observed in such mutants, it appears that the RAD50 gene product is involved in an early step in recombination which leads to the appearance of SSI's. The RAD52 gene product can then process the resulting intermediates. These results are consistent with our observation that a product of the RAD52 gene is a single-strand deoxyribonuclease. Models by us and others, propose double-strand breaks as possible intermediates in meiotic recombination; the RAD52 gene product could be a critical enzyme in their production and repair. We are currently investigating this.

This program represents a unique opportunity to examine mitotically identified DNA repair functions at the molecular level during meiosis. With the present system, we have been able to examine specific DNA changes during meiosis, the genetic control and at least one enzyme that is involved. We are expanding this work to examine the nature of the recombinational events and possible sites of recombination.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Little is known about the role of DNA repair mechanisms during the meiotic development of eukaryotes. With the techniques we have developed, yeast affords the opportunity to examine at both the genetic and the molecular level the importance of various repair systems during normal meiosis and following challenges by various mutagens during meiosis. The yeast system may also serve as a relevant model for understanding events in the germ lines of whole animals wherein, for technical reasons and lack of genetic systems, many of these studies cannot be conducted.

PUBLICATIONS

Game, J.C., T.J. Zamb, R.J. Braun, M. Resnick, and R.M. Roth (1980). The role of radiation (rad) genes in meiotic recombination in yeast. Genetics 94: 51-68.

Resnick, M.A., J.N. Kasimos, J.C. Game, R. Braun, and R.M. Roth (1981). Changes in DNA during meiosis in a repair-deficient mutant (rad52) of yeast. Science 212: 543-545.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60127-02 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Use of the Intra-sanguinous Host Mediated Assay to Study Organ-Specific Mutagenicity of Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Supervisory Microbiologist	CGTB NIEHS
	D. Pagano	Research Microbiologist	CGTB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The intrasanguinous host-mediated assay is being used to study the metabolism of chemicals to mutagenic products in different organs of mice. Bacteria or yeast cells are injected into the tail vein and can be recovered from the liver, kidneys, lungs and testes. Initial studies with control chemcials have been done.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The testing procedure used is the intra-sanguinous host-mediated assay. An indicator strain is injected into the tail vein of mice and the chemical being tested is given orally or by IP or IM injection. After in vivo incubation, the mice are sacrificed and the tester strain is recovered from various organs by excision, homogenization, dilution and plating of the homogenates onto complete plates for survival and minimal media plates for enumeration of mutant colonies.

MAJOR FINDINGS AND PROPOSED COURSE: Using dimethylnitrosamine (DMN) as the positive control, a number of compounds were tested. These included nitrosomorpholine (NM), nitrosopiperazine (NP), cyclophosphamide (CYCP), trichloroethylene (TCE), styrene, procarbazine (PCB), 4-chloromethylbiphenyl (4CMB), tris(2,3-dibromopropyl) phosphate (TRIS), dimethylaminoazobenzene (DAB), β-naphthylamine (BNA) and 2-aminoanthracene (2AA).

Looking at livers only and after 4 hours incubation (in vivo), 4CMB (285 mg/kg, IP), DAB(570 Mg/kg, IP), TRIS(4200 mg/kg, IP), styrene(1140 mg/kg, IP and gavage), TCE(285 mg/kg, IP and gavage), BNA(1.4 mg/kg, gavage) and 2AA(285 mg/kg, gavage) were not mutagenic to strain TA2410. NP(125 mg/kg, gavage) was also not mutagenic to TA2410 and G46. NM was mutagenic to strains TA2410(140 mg/kg) and TA100(71 mg/kg) when given by gavage and incubated only 1 hour in vivo, but not mutagenic to G46 when incubated 4 hours in vivo. PCB was a weak mutagen(571 mg/kg, IV, IP, gavage) for TA2410 after 4 hours incubation. CYCP(570 mg/kg) was not mutagenic to strain TA240 under any conditions tested.

Studies are planned for testing a series of structurally-related nitrosamines with organ-specific carcinogenicity in order to study the ability of this short-term test to detect target-organ specificity. Also, work on bacterial cell recovery from other organs will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The host-mediated assay provides the opportunity for detecting the effects of in vivo metabolism after chemical exposure by a number of different routes, using mutation induction in injected microorganisms as an endpoint. Additionally, the potential for determining target organ specificity adds a dimension to short-term testing that is currently only available in chronic long-term bioassays.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60128-02 CGTB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Collaborative Study to Test for "Genetic Drift" in Laboratory Stocks of Ames' Strains

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Co PI's:	E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
	M. Shelby	Geneticist	CGTB	NIEHS
	B. Margolin	Mathematical Statistician	BRAP	NIEHS
	K. Risko	Statistician	BRAP	NIEHS
	I. Robertson	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)
British Industrial Biological Research Association, United Kingdom

LAB/BRANCH
Cellular and Genetic Toxicology Branch, Biometry and Risk Assessment Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As part of a 38-laboratory international study our stocks of the Salmonella typhimurium tester strains were compared with reference strains in their response to a mutagen, 4-nitroquinoline-N-oxide. The results from all laboratories are being analyzed in order to determine the levels of agreement within and between laboratories for each Salmonella strain.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Each laboratory's own cultures and a set of reference cultures were tested with 4-NQO under the same conditions using a set protocol. Strains were also checked for known characteristics.

MAJOR FINDINGS: The data from the various laboratories are being analyzed and the responses are being compared. Preliminary evaluations show that the majority of laboratories do not show significant differences in control values between the in-house and reference cultures. Also, the variances around the control means of the two cultures may not be significantly different (with the exception of occasional outliers). More detailed analyses are in progress. Two European laboratories are also analyzing this data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will allow a determination of the extent to which stocks of Salmonella strains held in different laboratories have diverged from each other in their control responses and responses to a mutagen.

It will also demonstrate the extent of intra- and inter-laboratory variation and will provide information as to whether the variation seen is a function of the Salmonella culture used, or of other laboratory-related factors.

ARGONNE NATIONAL LABORATORY
Argonne, Illinois 60439
(YO1-CP-10205)

ARTHUR D. LITTLE, INC.
Cambridge, Mass.02140
(NO1-CP-15794)

MICROBIOLOGICAL ASSOCIATES
Bethesda, Maryland 20016
(NO1-CP-15758)

TITLE: Task I - Mammalian Cell Transformation Using Syrian Golden Hamster Embryo Cell Culture Using the Colony Transformation Endpoint

CONTRACTORS' PRINCIPAL INVESTIGATORS: Dr. E. Huberman (YO1-CP-10205)
Dr. A. Tu (NO1-CP-15794)
Dr. R. Kouri (NO1-CP-15758)

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: October 1, 1981

CURRENT ANNUAL LEVEL: YO1-CP-10205 = $325,000
NO1-CP-15794 = $277,681
NO1-CP-15758 = $189,885

PROJECT DESCRIPTION

OBJECTIVES: This project is a three-laboratory evaluation, using coded chemicals, of the Syrian hamster embryo oncogenic transformation assay for detection of potential chemical carcinogens. Initial objectives involve the development of a standardized test protocol, identification of the sources of intra- and inter-laboratory variability and establishment of interlaboratory reproducibility of the test system. Results of previous contract-supported studies and published results have shown that the SHE transformation assay detects chemical carcinogens. This project is one part of an effort to systematically evaluate and compare three assays for oncogenic transformation to determine which system may be most useful in identifying chemical carcinogens.

METHODS EMPLOYED: Syrian hamster embryo cells are collected, frozen, characterized for their response to known carcinogens, and then exposed to concentrations of the test chemical, based upon previous tests for toxicity. After 7-10 days, treated cultures are examined for foci of transformed cells.

MAJOR FINDINGS AND PROPOSED COURSE: For the first year of this study, the major goals include: 1) the standardization of the test protocol; 2) identification of key test reagents and materials; 3) selection of optimal lots of reagents and materials following preliminary testing; 4) acquisition of sufficient quantities of critical reagents (from identical sources) by all contract laboratories; and 5) tests of representative transformation positive and negative chemicals for toxicity and transformation to establish interlaboratory reproducibility of the methods. Each contract laboratory has the responsiblity of focusing on key components of the test system (e.g. identification of suitable frozen cell pools; identification of optimal serum and medium stocks). Progress has been made in all these areas, although some technical aspects require further evaluation before a final protocol can be defined. Preliminary toxicity and transformation assays of the standard chemicals are being performed in all three laboratories. The results of these independent tests will be the basis for determining the degree of interlaboratory reproducibility of the protocol. As soon as the major technical issues have been resolved and an acceptable degree of standardization achieved, all laboratories will begin to test coded chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of independent studies have shown a high correlation between the ability of chemicals which are known to induce tumors *in vivo* to induce oncogenic transformation in certain cultured mammalian cells. Such *in vitro* systems offer significant advantages in time and cost over animal bioassays for carcinogens. In addition, they often provide information for mechanistic inferences on the toxicity of chemicals. It is important that the use of such test systems include the application of standardized protocols which provide a high degree of interlaboratory reproducibility and an understanding of the biological limitations of the test system.

MICROBIOLOGICAL ASSOCIATES
Bethesda, Maryland 20016
(NO1-CP-15795)

NORTHROP SERVICES, INC.
Research Triangle Park, NC 27709
(NO1-CP-15796)

TITLE: Task II - Mammalian Cell Transformation using Syrian Hamster Embryo (SHE) Cells Infected with Simian Adenovirus (SA7)

CONTRACTORS' PRINCIPAL INVESTIGATORS: Dr. Leonard Schechtman (NO1-CP-15795)
Dr. George Hatch (NO1-CP-15796)

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch, and Judson Spalding, Ph.D. (Co-project Officer)

DATE CONTRACT INITIATED: October 1, 1981

CURRENT ANNUAL LEVEL: NO1-CP-15795 = $230,949
NO1-CP-15796 = $224,642

PROJECT DESCRIPTION

OBJECTIVES: This project is a dual laboratory evaluation using coded chemicals, of the SA7/SHE transformation assay system for detecting potential chemical carcinogens. Initial objectives involve: 1) the development of a standardized test protocol; 2) the identification of the sources of intra- and interlaboratory variability; and 3) the establishment of the interlaboratory reproducibility of the test system. Published results on the SA7/SHE transformation enhancement assay indicate that the system detects chemicals of known carcinogenic potential, and may be particularly useful in the identification of potential carcinogens from some specific chemical classes which are not easily detected in other assays for genetic toxicity. This project is one part of an effort to systematically evaluate and compare three assays for oncogenic transformation to determine which system may be most useful in identifying chemical carcinogens.

METHODS EMPLOYED: Primary cultures of SHE cells are prepared from pooled 13 day gestation embryos; transforming virus is obtained from standardized frozen stocks of SA7 with defined PFU/FFU ratio. Cells are infected with virus prior to or after treatment with doses of test chemical, that have been selected on the basis of previously determined toxicity. Cultures are scored for toxicity and transformed foci after 7-9 days of cultivation and the transformation frequency and enhancement ratio for each chemical is determined.

MAJOR FINDINGS AND PROPOSED COURSE: In the first year of this study, the major goals include: 1) standardization of the test protocol; 2) identification of key test reagents and materials; 3) selection of optimal lots of reagents and materials following preliminary testing; 4) acquisition of sufficient quantities of critical reagents (from identical sources) by both laboratories; and 5) tests of representative transformation positive and negative chemicals for toxicity and transformation to establish the interlaboratory reproducibility of the methods. Progress has been made in all of these areas, although some technical aspects require further evaluation before final decisions can be made. Preliminary toxicity tests with the standard chemicals are being performed in both laboratories. The results of these independent tests will be the basis for determining the degree of interlaboratory reproducibility of the protocol. As soon as the major technical

issues have been resolved and an acceptable degree of standardization achieved, both laboratories will begin to test coded chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of independent studies have shown a high correlation between the ability of chemicals which are known to induce tumors *in vivo* to induce oncogenic transformation in certain cultured mammalian cells. Such *in vitro* systems offer significant advantages in time and cost over animal bioassays for carcinogens. In addition, they often provide information for mechanistic inferences on the toxicity of chemicals. It is important that the use of such test systems include the application of standardized protocols which provide a high degree of inter-laboratory reproducibility and an understanding of the biological limitations of the test system.

BIOTECH RESEARCH LABS
Rockville, Maryland 20014
(NO1-CP-15807)

LITTON BIONETICS
Kensington, MD 20895
(NO1-CP-15797)

NORTHROP SERVICES, INC.
Research Triangle Park, NC 27709
(NO1-CP-15798)

TITLE: Task III - Mammalian Cell Transformation Retrovirus Infected Rat Cells

CONTRACTORS' PRINCIPAL INVESTIGATORS: Dr. R. Ting (NO1-CP-15807)
Dr. J. Poiley (NO1-CP-15797)
Dr. W. Suk (NO1-CP-15798)

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: October 1, 1981

CURRENT ANNUAL LEVEL: NO1-CP-15807 = $120,781
NO1-CP-15797 = $393,815
NO1-CP-15798 = $249,576

PROJECT DESCRIPTION

OBJECTIVES: This project is a three-laboratory evaluation, using coded chemicals, of the rat cells infected with the Rauscher leukemia virus ($2FR_4$50) oncogenic transformation assay for detection of potential chemical carcinogens. Initial objectives involve the development of a standardized test protocol, identification of the sources of intra- and interlaboratory variability and establishment of interlaboratory reproducibility of the test system. Results of previous contract-supported studies and published results have shown that this transformation assay detects chemical carcinogens. This project is one part of an effort to systematically evaluate and compare three assays for oncogenic transformation to determine which system may be most useful in identifying chemical carcinogens.

METHODS EMPLOYED: The infected ($2FR_4$50) and uninfected (2FRN) cell lines obtained from American Type Culture Collection were cultivated from passage 7. The cells are first exposed to chemicals to determine the toxicity and subsequently appropriate doses are applied and the cells are tested for transformation by the aggregation (survival) assay which detects the preferential ability of transformed cells to survive under the test conditions.

MAJOR FINDINGS AND PROPOSED COURSE: For the first year of this study, the major goals include: 1) the standardization of the test protocol; 2) identification of key test reagents and materials; 3) selection of optimal lots of reagents and materials following preliminary testing; 4) acquisition of sufficient quantities of critical reagents (from identical sources) by all contract laboratories; and 5) tests of representative transformation positive and negative chemicals for toxicity and transformation to establish interlaboratory reproducibility of the methods. Each contract laboratory has the responsiblity of focusing on key components of the test system (e.g. identification of suitable frozen cell pools; identification of optimal serum and medium stocks). Progress has been made in all these areas, although some technical aspects require further evaluation before a final protocol can be defined. Preliminary toxicity and transformation assays of the standard chemicals are being performed in all three laboratories. The results of these independent tests will be the basis for determining the degree of interlaboratory reproducibility of the protocol. As soon as the major

technical issues have been resolved and an acceptable degree of standardization achieved, all laboratories will begin to test coded chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of independent studies have shown a high correlation between the ability of chemicals which are known to induce tumors *in vivo* to induce oncogenic transformation in certain cultured mammalian cells. Such *in vitro* systems offer significant advantages in time and cost over animal bioassays for carcinogens. In addition, they often provide information for mechanistic inferences on the toxicity of chemicals. It is important that the use of such test systems include the application of standardized protocols which provide a high degree of interlaboratory reproducibility and an understanding of the biological limitations of the test system.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(Y01-ES-10061)

TITLE: Assay of Specific Sequence Transposition in Mammalian Cells

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Wen Yang

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: October 15, 1980

CURRENT ANNUAL LEVEL: $350,000

PROJECT DESCRIPTION

OBJECTIVES: The major aim is to derive specific experimental approaches and molecular probes for detection of gene sequence transposition in mammalian cells upon carcinogenic insults. Endogenous (germ-line associated) retroviral genomes of RFM/Un strain mice currently serve as experimental models for the study. Molecular probes, derived from molecularly cloned retroviral DNA, will be used to establish the specific gene loci in the normal cells as well as to detect potential new sites in neoplastic cells.

METHODS EMPLOYED: Normal and neoplastic RFM strain cells were cultured in vitro, with particular emphasis on hematopoietic tissues. Endogenous retroviruses are induced by chemical treatment of the cells and isolated viruses are characterized biologically and biochemically. The viruses are used to prepare total retroviral molecular probes as well as to prepare unintegrated viral DNA for molecular cloning purposes. Restriction endonuclease mapping and Southern gel blotting are used to characterize the viral DNA structure. DNA sequencing methods are used to determine the primary nucleotide structure of long terminal repeat sequence.

MAJOR FINDINGS AND PROPOSED COURSE: 1. By employing hamster/RFM mouse cell hybrids which segregate mouse chromosomes and Southern gel blotting of the DNA preparations from these somatic cell hybrid clones, it was found that the single ecotopic retrovirus of RFM/Un mice is located on chromosome 5. 2. RFM/Un endogenous ecotopic virus has been molecularly cloned in a λ phage as well as in plasmids; LTR sequence of a viral DNA clone has been determined. 3. Preliminary results indicated additional integration of ecotopic retroviral genomes or a myeloid leukemic cell line. Ongoing and future studies will include subcloning of various gene regions of the RFM/Un endogenous ecotopic viral genome, examination of possible additional retrovirus integration in radiation-induced primary myelogenous leukemias, determination of endogenous retroviral provirus in the preleukemic stage of leukemias and future characterization of the restrictive mechanism of RFM/Un mice against horizontal spread of the endogenous retroviruses.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is considerable evidence that the genetic effects of toxic or carcinogenic agents involves alteration of specific gene elements or genetic mechanisms which are regulatory in nature. Particular emphasis has been placed on transposable genes

and mechanisms of gene transposition in mammalian cells. Information concerning these mechanisms and their relationship to neoplastic transformation is a prerequisite for the technical development of an assay system which can utilize these advances.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, TN 37830
(Y01-CP-10203)

TITLE: Chromosome Aberrations and Sister-Chromatid Exchanges in Human Lymphocytes

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. R. Julian Preston

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $100,300

PROJECT DESCRIPTION

OBJECTIVES: The first objective of this project is to develop a standardized protocol by which the frequencies of chromosome aberrations and sister-chromatid exchanges can be accurately and reproducibly determined in human lymphocytes. This will require the demonstration of reproducible results between two laboratories. The second objective is a better understanding of the frequencies of these cytogenetic endpoints and the variables (e.g. sex, age, race, time) that may affect their frequencies. The long range goal is to provide a step toward improving our ability to design and interpret human cytogenetic studies.

METHODS EMPLOYED: Lymphocyte cultures are established from heparinized whole blood samples within 24 hrs of collection. Culture medium containing 5-bromodeoxyridine is used to determine the frequency of first, second, and third division cells at harvest time (48 hrs for aberrations, 56 hrs for SCE) and to provide BU-substituted chromosomes for the scoring of SCE. For chromosome aberrations, 200 cells per individual are scored and 50 are scored for SCE.

MAJOR FINDINGS AND PROPOSED COURSE: It has been demonstrated that blood samples can be stored at 4°C, shipped by airfreight and cultured within 24 hrs without a significant loss in growth potential of the lymphocytes. A reduced quality of chromosome differentiation for SCE analysis was noted in shipped samples. This appeared to be a result of shipping/handling and not of storage since the phenomenon was not observed in cells maintained at 4°C for 24 hrs at the site of collection. Reproducibility of scoring both SCE and aberrations has been demonstrated at the two laboratories. The potential for differences in SCE frequencies as a result of serum batches was also observed. Additional data are being obtained in the initial subjects of the study to further demonstrate the utility of the protocol. The feasibility of scoring both aberrations and SCE on the same BU substituted slides is being pursued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Monitoring peripheral lymphocytes for cytogenetic damage offers one of the only available means for detecting exposures of individuals or populations to genotoxic agents. A better understanding of the variability in frequences of chromosome aberrations and SCEs and the sources of variability will, along with standardized protocols with demonstrated interlaboratory reproducibility, permit better design and interpretation of future human cytogenetic monitoring and surveillance studies.

BROOKHAVEN NATIONAL LABORATORY
Upton, Long Island, NY 11973
(YO1-CP-10204)

TITLE: Chromosome Aberrations and Sister-Chromatid Exchanges in Human Lymphocytes

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Michael A. Bender

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $149,957

PROJECT DESCRIPTION

OBJECTIVES: The first objective of this project is to develop a standardized protocol by which the frequencies of chromosome aberrations and sister-chromatid exchanges can be accurately and reproducibly determined in human lymphocytes. This will require the demonstration of reproducible results between two laboratories. The second objective is a better understanding of the frequencies of these cytogenetic endpoints and the variables (e.g. sex, age, race, time) that may affect their frequencies. The long range goal is to provide a step toward improving our ability to design and interpret human cytogenetic studies.

METHODS EMPLOYED: Lymphocyte cultures are established from heparinized whole blood samples within 24 hrs of collection. Culture medium containing 5-bromodeoxyridine is used to determine the frequency of first, second, and third division cells at harvest time (48 hrs for aberrations, 56 hrs for SCE) and to provide BU-substituted chromosomes for the scoring of SCE. For chromosome aberrations, 200 cells per individual are scored and 50 are scored for SCE.

MAJOR FINDINGS AND PROPOSED COURSE: The feasibility of storing, shipping, and successfully culturing blood samples was demonstrated as was the reproducibility of scoring common slides in the two participating laboratories. Aberration and SCE data have been obtained on more that 15 subjects with no remarkable results to date. Determinations of first, second, and third division cells in 48 hr cultures (the fixation time normally chosen to insure scoring of first division cells) produced somewhat surprising results in that an average of 16% of cells are in their second mitosis at this time. Additional data are being gathered to refine the protocol and insure the reproducibility of results between the two participating labs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Monitoring peripheral lymphocytes for cytogenetic damage offers one of the only available means for detecting exposures of individuals or populations to genotoxic agents. A better understanding of the variability in frequences of chromosome aberrations and SCEs and the sources of variability will, along with standardized protocols with demonstrated interlaboratory reproducibility, permit better design and interpretation of future human cytogenetic monitoring and surveillance studies.

LITTON BIONETICS INCORPORATED - Kensington, Maryland 20795
(NO1-CP-65853)

TITLE: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening.

CONTRACTOR'S PROJECT OFFICER: Dr. Brian Myhr

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1976

CURRENT ANNUAL LEVEL: $216,769

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the usefulness and reliability of an in vitro mutagenesis assay system using L5178Y mouse lymphoma cells ($TK^{+/-}$ locus) as a prescreen for potential chemical carcinogens.

METHODS: The mouse lymphoma assay is being run in a collaborative study between this lab and SRI International. In the protocol which has been set up for these labs, mouse lymphoma L5178Y $TK^{+/-}$ cell cultures are exposed to a test chemical for four hours. These cells then undergo a two day expression period. Following this, $3x\ 10^6$ cells are seeded in soft agar with selection medium (TFT) and the resistant (mutant) colonies are counted after a ten day incubation period. The cloning efficiency is determined by plating 600 cells from the cell suspension in nonselective medium and taking the ratio of surviving clones to initial cells after a ten day incubation period. Acceptability criteria have been set up as a quality control. These criteria reject experiments which lack a significant number of acceptable dose sets, or show deviant solvent or positive controls. Initially experiments are run with no activation and with activation provided by Aroclor 1254 induced liver homogenates from male Fischer 344 rats (S-9 activation). Experimental results are determined based on a set of statistics which estimates the variance of the mutant frequency. Overall assay results for a compound are determined by looking at sets of experimental results within activation conditions (test results).

MAJOR FINDINGS AND PROPOSED COURSE: This project had proceeded in four phases; the first three phases were concerned with the development of the L5178Y $TK^{+/-}$ mouse lymphoma in vitro forward mutation assay. The contract is presently in phase four, the testing of coded compounds for the purpose of determining reproducibility. To date approximately sixty compounds are in various stages of testing, forty of which have been completed, include ultimate carcinogens, procarcinogens, noncarcinogenic analogs and chemicals of uncertain carcinogenicity. Evaluations by the two laboratories have shown excellent between-lab agreement (greater than 90%) and results have also shown a high correlation between mutagenic response and the carcinogenic properties of the chemicals. Methods for quality control, data analysis and statistical evaluation have been developed. Based on the results from this work, this assay is ready to be utilized in the testing mode. In the future more coded compounds will be tested, providing a comparison to other in vitro assays. The planned termination date of this contract is September 29, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal carcinogenesis test systems, there is a need for short-term tests, such as the mouse lymphoma mutagenesis assay system, that may be used together with other tests for the initial evaluation of chemicals for possible carcinogenic potential.

SRI INTERNATIONAL - Menlo Park, California 94025
(NO1-CP-65854)

TITLE: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis

CONTRACTOR'S PROJECT OFFICER: Dr. Ann Mitchell and Dr. Collette Rudd

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1976

CURRENT ANNUAL LEVEL: $237,710

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the usefulness and reliability of an in vitro mutagenesis assay system using L5178Y mouse lymphoma cells ($TK^{+/-}$ locus) as a prescreen for potential chemical carcinogens.

METHODS: The mouse lymphoma assay is being run in a collaborative study between this lab and Litton Bionetics. In the protocol which has been set up for these labs, mouse lymphoma L5178Y $TK^{+/-}$ cell cultures are exposed to a test chemical for four hours. These cells then undergo a two day expression period. Following this $3x10^6$ cells are seeded in soft agar with selection medium (TFT) and the resistant (mutant) colonies are counted after a ten day incubation period. The cloning efficiency is determined by plating 600 cells from the cell suspension in nonselective medium and taking the ratio of surviving clones to initial cells after a ten day incubation period. Acceptability criteria have been set up as a quality control. These criteria reject experiments which lack a significant number of acceptable dose sets, or show deviant solvent or positive controls. Initially experiments are run with no activation and with activation provided by Aroclor 1254 induced liver homogenates from male Fischer 344 rats (S-9 activation). Experimental results are determined based on a set of statistics which estimates the variance of the mutant frequency. Overall assay results for a compound are determined by looking at sets of experimental results within activation conditions (test results).

MAJOR FINDINGS AND PROPOSED COURSE: This project had proceeded in four phases; the first three phases were concerned with the development of the L5178Y $TK^{+/-}$ mouse lymphoma in vitro forward mutation assay. The contract is presently in phase four, the testing of coded compounds for the purpose of determining reproducibility. To date approximately sixty compounds are in various stages of testing, forty of which have been completed, include ultimate carcinogens, procarcinogens, noncarcinogenic analogs and chemicals of uncertain carcinogenicity. Evaluations by the two laboratories have shown excellent between-lab agreement (greater than 90%) and results have also shown a high correlation between mutagenic response and the carcinogenic properties of the chemicals. Methods for quality control, data analysis and statistical evaluation have been developed. Based on the results from this work, this assay is ready to be utilized in the testing mode. In the future more coded compounds will be tested, providing a comparison to other in vitro assays. The planned termination date of this contract is September 29, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal carcinogenesis test systems, there is a need for short-term tests, such as the mouse lymphoma mutagenesis assay system, that may be used together with other tests for the initial evaluation of chemicals for possible carcinogenic potential.

INVERESK RESEARCH INTERNATIONAL - Edinburg, Scotland
(NO1-CP-75858)

TITLE: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

CONTRACTOR'S PROJECT DIRECTOR: Dr. Douglas McGregor

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: December 29, 1976

CURRENT ANNUAL LEVEL: No Cost Extension

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the Salmonella microbial mutagenicity assay for its ability to measure the mutagenic activity of chemicals and to develop new approaches to measuring toxicity in this assay.

METHODS: Coded chemicals were systematically tested using a standard microbial protocol which used five strains of Salmonella tymphimurium (TA1535, TA1537, TA1538, TA98 and TA100) in the presence and absence of metabolic activation conditions. The S-9 preparations used for metabolic activation were derived from the livers of non-induced and Aroclor 1254 induced mice, rats and hamsters. A major impediment to developing a consistent method of data evaluation based on statistical support is the lack of toxicity information in this assay. The laboratory is monitoring toxicity by using an automatic colony counter to count the number of microcolonies on each plate.

MAJOR FINDING AND PROPOSED COURSE: The test has been completed on approximately 130 coded compounds (some coded compounds being the same chemical under different codes). All have been decoded. The testing results have shown excellent qualitative agreement with results reported in the literature. The few exceptions seem to be due to chemicals whose mutagenic dose, found in the literature, was above the maximum dose that was called for in the standard protocol. This assay has been found to be quite reproducible in this laboratory, as indicated by responses for chemicals tested at different times under the same code. A good qualitative correlation with other contract laboratories has also been observed. Initial results from the use of counting microcolonies to measure toxicity are encouraging. The proposed course of this contract is to measure the toxic and mutagenic response of test chemicals and to compare the measurements of toxicity using the microcolony technique with that of the "filler cell" technique developed on another contract. This contract will terminate in December 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of mutagenic agents in the environment is a prerequisite for the elimination of such agents from our surrounding. The present study seeks to develop and evaluate rapid, simple and inexpensive microbial procedures to be used as preliminary screens for chemical carcinogens.

ARTHUR D. LITTLE, INCORPORATED - Cambridge, Mass. 02140
(NO1-CP-55711)

TITLE: Development of Detailed Methods and Protocols of Carcinogenesis Screening Using Cell Culture Asssays- Task II - BALB/c-3T3 Cells.

CONTRACTOR'S PROJECT DIRECTOR: Dr. Andrew Sivak

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: No Cost Extension

PROJECT DESCRIPTION

OBJECTIVES: To develop and standardize methods for performing in vitro transformation assays with BALB/c-3T3 cells.

METHODS: The BALB/c-3T3 neoplastic transformation assay was used to measure the ability of chemical agents to induce alterations in a population of cells (derived from mouse embryo fibroblasts) from a pattern of controlled monolayer growth to one exhibiting foci of disorientation, and piled up growth against a background monolayer. Toxicity is used to determine the highest dose (10-20% cell survival) at which a chemical will be tested. A chemical which induced a doubling or higher transformation frequency as compared to the controls was considered positive.

MAJOR FINDING AND PROPOSED COURSE: A number of factors that affect the quantitative assesments of transformation were examined. The results showed that the survival of cells in mass culture is invariably greater at toxic doses than one would expect from extrapolation of direct cloning data. Also, the number of cells surviving until the end of treatment was dependent on the source of insult and was not reflected in the direct cloning data. These observations suggest that the use of direct cloning data to estimate the number of cells at risk in the transformation assay grossly underestimates the actual population of cells esposed to the test chemical.

A study was conducted to correlate anchorage-independent growth and in vivo tumorigenicity with progression of Type III foci in cell passage. It was found that the transformed population (Type III foci) acquired the ability to grow in soft agar at the earliest passage. However, although most Type III cell populations grew tumors in irradiated syngeneic animals, the cell populations did not exhibit quantitative correlation of growth in soft agar and tumorigenicity. Tumorigenicity of transformed populations, contrary to growth in soft agar, was dependent on cell passages in culture. This suggests that populations of Clone I-13 contain premalignant cells, which develop tumorigenic potential after extensive passage in culture. Due to the fact that growth in soft agar was not found to be a consistent marker of oncogenicity for BALB/c-3T3 cells another proposed marker, basal cathepsin B activity, was examined. This marker however was not found to correlate with either the ability of populations to grow in soft agar or to grow tumors in animals.

In comparisons run between cells of the I-13 clone and other cells there were varied results. Cells from the I-11 clone showed no spontaneous background transformation frequency, but were significantly less sensitive to chemical treatment. Clone I-11 cells also showed little effect when enhancement with a tumor promoter was attempted. A comparison of the metabolism of MCA by I-13 and C3H-10½ cells showed differences in kinetics between the two. However, no simple relationship between the rate of MCA metabolism and tranformation by MCA metabolism and tranformation by MCA could be obtained. The testing of seventy coded samples which have been completed using the BALB/c-3T3 system has revealed a good correlation with in vivo carcinogenic activity of the chemicals tested. Aromatic amines, nitrosamines and synthetic hormones such as diethylstilbestrol showed no transforming activity. Studies done to provide the BALB/c-3T3 cells with exogenous activation showed some success in that aroclor induced adult rat hepatocytes increased the sensitivity of the system to two procarcinogens (MCA and 2-AF). Hepatocytes, however, proved to be very toxic to the target cells at all cell densities, and in some experiments hepatocytes alone increased the transformation frequency. Addition of mouse S-9 showed no beneficial effects in the activation of the system. Because of program needs, this laboratory has resumed the testing compounds and is in the process of evaluating compounds of direct program interest. The planned termination date for this contract is September 29, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The carcinogenic risk to man from chemical agents now present or being introduced in the environment is largely unknown. The task of testing these materials by the classic chronic animal exposure protocols is formidable and prohibitive with respect to resources. While the short-term screening tests for mutagenicity with microorganisms are more reasonable in cost, they are at best only indicative of some genomic alterations and do not necessarily reflect the process of carcinogenesis. The cell culture systems under study are being used to provide standardized procedures to examine chemicals for carcinogenicity in short-term systems. Further, these cell culture systems may allow a more detailed explanation of the sequential cellular processes occurring in the development of neoplastic disease.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(Y01-ES-10067)

TITLE: Potential Hazard from Chemically Induced Transmitted Gene Mutations Using the Specific Locus Method in Mice

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Liane B. Russell

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: April 15, 1981

CURRENT ANNUAL LEVEL: $382,821

PROJECT DESCRIPTION

OBJECTIVES: The first objective of this project is to investigate chemically-induced mutation processes in mice by using the powerful germ-cell mutagen N-ethyl-N-nitrosourea (ENU). This compound is sufficiently mutagenic to permit the thorough study of cell stage specificity, dose response curves, and effects on both male and female germ cells. The second objective is to test five chemicals of environmental significance for germ-cell mutagenicity using the morphological specific locus assay.

METHODS EMPLOYED: Induced mutant frequencies are determined by administering ENU or the test chemical to one parent, usually the male, that is homozygous wild-type for seven morphological markers (primarily coat color markers). The treated parent is mated to the untreated parent which is homozygous recessive at the same seven loci. Mutant offspring are detected at 3-4 weeks of age as those exhibiting a visible recessive trait among the normal offspring that appear as wild-type.

MAJOR FINDINGS AND PROPOSED COURSE: A dose response curve has been determined for ENU induced mutations in spermatogonia at eight doses from 0 to 250 mg/kg. There is a strong indication that the curve is not linear with points below 100 mg/kg falling below a straight line fit to the control value. Additional data will be obtained at doses below 100 mg/kg. Fractionated dose experiments have shown that 10 injections of 10 mg/kg ENU yield a much lower frequency of mutants than a single injection of 100 mg/kg indicating an efficient repair of ENU induced damage. Experiments are underway to further investigate male post meiotic germ-cell stages, effects on female germ cells, and age effects. Dosimetry studies using tritiated ENU show a much higher level of ethylation in the liver than in the testes. In testicular DNA, there appears to be a linear relationship between dose administered intraperitoneally and ethylation from 10 to 100 mg/kg ENU. Tests are presently being designed to evaluate the germ-cell mutagenicity of 1,2-dibromo-3-chloropropane (96-12-8) and hexamethyl-phosphoramide (680-31-9).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is known that: 1) Humans are exposed to chemicals; 2) chemicals can induce mutations; and 3) mutations are the basis for a significant portion of human disease. This

project is designed to contribute to the protection of human health through further understanding of the process of induced mutations in mammalian germ cells, assessing the mutagenicity of selected, environmentally significant chemicals, and contributing data for use in genetic risk estimation efforts.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC.
Lexington, KY 40503
(NO1-CP-15789)

TITLE: Chromosome Damage Testing in Chinese Hamster Ovary Cells

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. P. S. Sabharwal

PROJECT OFFICERS (NIEHS): Michael D. Shelby, Ph.D., and Errol Zeiger, Ph.D., Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $216,756

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to test 20 coded chemicals for the induction of chromosome aberrations and sister-chromatid exchanges in Chinese hamster ovary cells.

METHODS EMPLOYED: Cultured Chinese hamster ovary cells are exposed to coded test chemicals in the presence and absence of a metabolic activation mixture (Aroclor 1254-induced rat liver S9). The frequency of chromosome aberrations and sister-chromatid exchanges are then determined in the treated cell population as well as the solvent and positive controls. Effects are determined for at least 3 doses of the test chemical. At each dose, 50 cells are scored for SCE and 100 cells for aberrations.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor completed Phase I of the contract by demonstrating the ability to conduct the CHO cytogenetic assay. This phase required the testing of choline chloride, quinoline, and maleic hydrazide. In Phase II of the contract, five coded test chemicals have been tested.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Test data from the CHO cytogenetic assay provide additional information on the genotoxic profile of chemicals selected for testing by the National Toxicology Program. These data are used to determine additional testing needs and, along with other short-term test data, to predict the potential for harmful effects in vivo.

RESEARCH TRIANGLE INSTITUTE
Research Triangle Park, NC 27709
(NO1-ES-2-5012)

TITLE: Mouse Electrophoretic Germinal Mutation Test Development

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Susan E. Lewis

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE INITIATED: December 1, 1981

CURRENT ANNUAL LEVEL: $394,428

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this contract are: 1) to investigate chemically induced mutation processes in mouse germ cells by studying cell stage specificity in both sexes and establishing a dose-response curve in spermatogonia using the mutagen, N-ethyl-N-nitrosourea (ENU); and 2) testing 3 environmentally significant chemicals for germ cell mutagenicity.

METHODS EMPLOYED: Induced mutant frequencies are determined by treating one parent (C57Bl/6J or DBA/2J), usually the male, with ENU or a test chemical and then mating to the alternate strain to obtain progeny. Blood and kidney samples are taken from the F_1 progeny and tissue preparations of these samples are subjected to starch gel electrophoresis. After appropriate staining, the electrophoretic patterns of 21 proteins are observed on the gels and altered mobility patterns or missing bands are noted as variants. Breeding tests with the animals from which the altered proteins were obtained, along with additional electrophoretic analyses, are used to confirm or refute the mutational basis of the variants.

MAJOR FINDINGS AND PROPOSED COURSE: At this early stage of the project, ENU treatments have been carried out and matings begun for: 1) post meiotic germ cells in both C57Bl/6J and DBA/2J males; 2) 200 mg/kg spermatogonial dose point; and 3) 250 and 100 mg/kg females. Insufficient progeny have been screened to draw conclusion in any of these studies. However, among 250 mg/kg treated C57Bl/6J females, litters were obtained from matings beyond 3 weeks post-treatment. The 100 mg/kg females produced litters from matings during the first week following treatment. No electrophoretic variants have been obtained from these 100 mg/kg females but one apparent dominant coat color female mutant was detected among 172 progeny. The nature of this variant will be determined when she is old enough to mate. A stain specific for Es-10 is being adapted for use in the screening system and, if incorporated, will increase the number of loci screened to 22.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: This project is designed to contribute to the assessment of chemical toxicity by: 1) testing chemicals for the ability to induce mutations in mammalian germ cells; 2) providing data for use in human genetic risk estimation; and 3) contributing to an understanding of the process of induced mutations in mammalian germ cells.

OAK RIDGE ASSOCIATED UNIVERSITIES
Oak Ridge, TN 37830
(Y01-CP-10206)

TITLE: Evaluation and Application of an in vivo Mouse Assay for Chemically Induced Sister-Chromatid Exchanges and Chromosome Aberrations

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Alfred F. McFee

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $162,078

PROJECT DESCRIPTION

OBJECTIVES: This project is being conducted in two laboratories, first, to develop and assess a testing protocol for the simultaneous determination of chemically induced chromosome aberrations and sister-chromatid exchanges in mouse bone marrow cells. Once an acceptable protocol is developed, approximately 20 chemicals will be tested for in vivo mutagenicity over a two-year period. Hybrid mouse strain $B6C3F_1$, the same strain used in the NTP cancer bioassay, is being used so that a more direct comparison can be made between the cytogenetic and carcinogenic effects of test chemicals.

METHODS EMPLOYED: In the preliminary phases of the study, $B6C3F_1$ male mice, 8-10 weeks old, are treated by intraperitoneal injection with the study compounds. Chromosomal aberrations are determined in bone marrow cell preparations. Sister-chromatid exchange frequencies and cell proliferation kinetics are determined through 5-bromodeoxyuridine (BU) substituted chromosomes.

MAJOR FINDINGS AND PROPOSED COURSE: A new method of coating BU pellets was developed. Partially coating pellets with paraffin has resulted in prolonged absorption of BU and improved differentiation of chromatids in second and third division cells following BU pellet implant. Smaller pellets (25 mg vs. 55 mg) were tested but found unsatisfactory. Studies are underway to determine bone marrow cell proliferation kinetics. Mitomycin C and dimethylbenzanthracene are being used as reference mutagens to investigate effects of cell cycle delay on chromosome aberration and SCE frequencies as well the effect of the relative times of administration of BU and mutagen (i.e. mutagen injected 1 hr before, 1 hr after and 8 hr after BU).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Chromosome aberrations and sister-chromatid exchanges are both endpoints associated with the induced genetic effects of many chemical mutagens and carcinogens. As such, these endpoints are potentially important as predictors of chemical genotoxicity, particularly when conducted in whole mammals where conditions for metabolism, distribution, etc., are more reflective of the human situation than are in vitro studies. The studies as conducted provide direct evidence of genotoxic effects in lab mammals, an effect that can, when necessary, be compared to similar effects in exposed humans. Further, the studies are being

carried out in the mouse strain used in the cancer bioassay program, and, hence, will permit a more direct comparison of induced somatic-cell genetic effects with carcinogenicity results. In addition to providing a screen for carcinogens, such comparisons may permit a better understanding of the relationship between induced cytogenetic effects and induced cancer.

BROOKHAVEN NATIONAL LABORATORY
Upton, Long Island, NY 11973
(YO1-CP-10207)

TITLE: Evaluation and Application of an in vivo Mouse Assay for Chemically Induced Sister-Chromatid Exchanges and Chromosome Aberrations

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Raymond Tice

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $169,306

PROJECT DESCRIPTION

OBJECTIVES: This project is being conducted in two laboratories, first, to develop and assess a testing protocol for the simultaneous determination of chemically induced chromosome aberrations and sister-chromatid exchanges in mouse bone marrow cells. Once an acceptable protocol is developed, approximately 20 chemicals will be tested for in vivo mutagenicity over a two-year period. Hybrid mouse strain $B6C3F_1$, the same strain used in the NTP cancer bioassay, is being used so that a more direct comparison can be made between the cytogenetic and carcinogenic effects of test chemicals.

METHODS EMPLOYED: In the preliminary phases of the study, $B6C3F_1$ male mice, 8-10 weeks old, are treated by intraperitoneal injection with the study compounds. Chromosomal aberrations are determined in bone marrow cell preparations. Sister-chromatid exchange frequencies and cell proliferation kinetics are determined through 5-bromodeoxyuridine (BU) substituted chromosomes. BU is administered by either pellet implant or tail vein infusion.

MAJOR FINDINGS AND PROPOSED COURSE: In the first half-year of this study, mitomycin C and cyclophosphamide were used to study bone marrow cell proliferation kinetics and the effect of mutagen time-of-administration relative to BU administration. Preliminary data have revealed anticipated dose response curves and cell cycle delays. A computerized data management system has been developed and is being used for storage of experimental details and storage and analysis of data. Cell proliferation studies will be completed as will the mutagen time-of-administration studies. Comparisons of results using BU pellets and tail vein infusions of BU will be conducted with cyclophosphamide and mitomycin C.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Chromosome aberrations and sister-chromatid exchanges are both endpoints associated with the induced genetic effects of many chemical mutagens and carcinogens. As such, these endpoints are potentially important as predictors of chemical genotoxicity, particularly when conducted in whole mammals where conditions for metabolism, distribution, etc., are more reflective of the human situation than are in vitro studies. The studies as conducted provide direct evidence of genotoxic effects in lab mammals, an effect that can, when necessary, be compared to similar effects in exposed humans. Further, the studies are being

carried out in the mouse strain used in the cancer bioassay program, and, hence, will permit a more direct comparison of induced somatic-cell genetic effects with carcinogenicity results. In addition to providing a screen for carcinogens, such comparisons may permit a better understanding of the relationship between induced cytogenetic effects and induced cancer.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH, FDA
Jefferson, AR 72079
(YO1-ES-20077)

TITLE: CGT Rapid Test Response - Component 1: DNA Damage Assay in Mammalian Cells

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Daniel Casciano

PROJECT OFFICER (NIEHS): Judson W. Spalding, Ph.D., Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: December 31, 1981

CURRENT ANNUAL LEVEL: $77,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are: 1) to detect chemically-induced DNA damage/repair measured by the incorporation of labeled thymidine into cellular DNA using an autoradiographic technique; and 2) to test approximately 25 coded National Toxicology Program (NTP) chemicals for the induction of unscheduled DNA synthesis in primary rat hepatocytes. This short-term test comprises one of five test components used to characterize the genotoxic activity of chemicals.

METHODS EMPLOYED: Primary hepatocytes are isolated following an in situ perfusion of rat livers with collagenase according to a standard protocol. Isolated cells are allowed to attach to coverslips for 1-2 hours, and they are then exposed for 18-24 hours to a test chemical over an appropriate concentration range in the presence of ^{3}H-thymidine. After incubation, the cells are processed for subsequent autoradiographic examination. The cells are stained and grains in the emulsion over the nuclei and cytoplasm are counted either visually or with an electronic counter.

MAJOR FINDINGS AND PROPOSED COURSE: Fourteen coded chemicals have been assigned for test and all 14 are in various stages of the test procedure. Another 12 chemicals of interest to the NTP will be selected for assignment to this assay. The expiration date of this agreement is November 29, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The Cellular and Genetic Toxicology Branch has the responsibility to provide short-term test information which can be utilized by the experimental design groups and in the ranking process for establishing the priority of chemicals to be entered into long-term carcinogenicity bioassays. The CGTB has developed a short-term test program that includes five broad classes of in vitro and in vivo short-term tests that will provide a comprehensive assessment of the capability of chemicals to effect mutation, chromosomes and DNA damage. The UDS assay in isolated rat hepatocytes is an integral part of this testing program and detects specifically the ability of chemicals to cause DNA damage and elicit a DNA repair process. This assay is useful in characterizing one mechanism by which chemicals can express genotoxic activity and potential.

ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, NC 27711
(YO1-ES-20079)

TITLE: CGT Rapid Test Response - Component II

WORK BEING PERFORMED AT THE FOLLOWING CONTRACT LABORATORIES:

SRI, International	Northrop Services, Inc.	Litton Bionetics, Inc.
(Dr. David Jones, PI)	(Dr. George Hatch, PI)	(Dr. John Rundell, PI)

PROJECT OFFICER (NIEHS): Judson W. Spalding, Ph.D., Cellular and Genetic Toxicology Branch

PROJECT OFFICER (EPA): Stephen Nesnow, Ph.D., Experimental Toxicology Division HERL

DATE CONTRACT INITIATED: December 29, 1981

CURRENT ANNUAL LEVEL: $970,700

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to test the nineteen 1982 bioassay-candidate chemicals and a limited number of other priority chemicals in a complementary group of short-term tests. These short-term test components have been selected to provide multiple test systems and endpoints for determining the ability of chemicals to directly damage DNA and/or alter gene expression. The results from these tests will contribute substantially to a data base which will permit an evaluation of the genotoxic effects of the bioassay-candidate chemicals prior to the initiation of chronic studies.

METHODS EMPLOYED: The test components were selected on the basis that the basic categories of genotoxic effects could be detected, and that the test protocols had been subjected to some form of evaluation or validation. The chemicals selected for test are coded and distributed for testing in the following five test components: 1) the "L5178Y TK$^{+/-}$ mouse lymphoma forward mutation" assay; 2) the "in vitro transformation of Balb/C 3T3 cell" assay; 3) the "enhancement of DNA virus transformation of Syrian hamster embryo cells by chemical test agents and Simian adenovirus SA7" assay; 4) the "host activated (in vivo - in vitro) hepatocyte DNA repair" assay; and 5) the "unscheduled DNA synthesis in rat liver primary cell culture" assay. When possible, chemicals submitted for testing will be taken from the same chemical lot that has been prepared for the bioassay. Where appropriate, protocols have been designed to provide dose-response data.

MAJOR FINDINGS AND PROPOSED COURSE: All 19 of the bioassay-candidate chemicals have been assigned for test in the five test-component assays. Nearly one-half of these chemicals are currently in some phase of testing in each of the test components. As the tests on the bioassay-candidate chemicals are completed, other chemicals selected because of their special interest to the NTP program, will be assigned. A data management system is being developed that will include a chemical tracking system to provide pertinent information on the chemicals assigned for genotoxicity testing in the CGTB. The expiration date of this agreement is December 30, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Cellular and Genetic Toxicology Branch has the responsibility for providing short-term test support to the carcinogenicity bioassay program. A variety of short-term tests have been proposed to predict the potential carcinogenicity of chemicals. Up to now, current data from short-term tests have not been sufficient to predict the potential carcinogenicity of those chemicals submitted for test in the two-year rodent bioassay. The short-term assays described in this project are included in three of the five broad classes of in vitro short-term tests selected by CGTB to characterize the genotoxicity potential of chemicals. The information obtained from this "Rapid in vitro Test" capability may be utilized by experimental design groups and in the ranking process for establishing the priority of chemicals to be entered into the long-term carcinogenicity assays.

1. UNIVERSITY OF CALIFORNIA - Berkeley, California
(NO1-ES-1-5002)

2. TECHNISCHE HOCHSCHULE - Darmstadt, Germany
(NO1-ES-1-5005)

TITLE: Development of Yeast Aneuploidy Test System

CONTRACTOR'S PROJECT DIRECTOR: 1. Seymour Fogel, Ph.D.
2. Fritz Zimmermann, Ph.D.

PROJECT OFFICER: Michael A. Resnick, Ph.D., Research Geneticist

DATE CONTRACT INITIATED: 1. July 1, 1981
2. July 1, 1981

OBJECTIVES: The purpose of these contracts is to develop a system with the yeast Saccharomyces cerevisiae which will enable the rapid screening of agents that induce aneuploidy during meiotic development and mitotic growth. In addition, the system(s) will enable a comparison of the effects of agents in terms of the induction of recombination and mutation as well as aneuploidy. The yeast aneuploid test system will become an integral component in the battery of tests used to detect genetically active agents. During the course of this work, over 100 chemicals are expected to be tested.

METHODS EMPLOYED: In the development of a meiotic aneuploidy test system, the contractors will devise a means for following chromosome gain among the haploid products of meiosis. The sytem will rely on screening for differences in gene dosage. For the case of aneuploidy among mitotically growing cells, a system will be devised based upon chromosome loss and selection of recessive resistance. With both the meiotic and the mitotic test systems that will be developed, the contractors will determine the most advantageous methods for a rapid screen of chemicals. The methods for testing will be based on results with a series of positive controls and coded chemicals supplied by NIEHS. After a protocol has been determined, it will be validated by screening a number of coded chemicals.

MAJOR FINDINGS AND PROPOSED COURSE: The meiotic test is being developed with several genetic markers which will enable a clear discrimination between cells that are aneuploid or false positive. The strains being used will also enable the detection of recombination events. The mitotic system is a refinement of a previous published system in that it enables a clearer discrimination against false positives. Conditions are being developed for reproducibly identifying true aneuploid as opposed to recombinational events.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aneuploidy contributes significantly to the genetically based disease burden in human populations with approximately 0.4% of live births exhibiting abnormal chromosome numbers. A large fraction of spontaneous

abortion in humans and certain serious genetic diseases (e.g., Down's syndrome) are caused by aneuploidy. Aneuploidy has also been implicated in some steps in tumor promotion. A few chemicals are known to induce aneuploidy specifically in test systems; however there is in fact, no reliable, well-developed rapid screen to detect such agents on a large scale. A yeast test system will enable the future rapid screening of chemicals and agents 1) that induce gross chromosomal changes which would not be identified as mutagenic in microbial test systems, and/or 2) cause changes in chromosomal number in addition to being mutagenic.

BIOMEDICAL SCIENCES DIVISION
LAWRENCE LIVERMORE NATIONAL LABORATORY
UNIVERSITY OF CALIFORNIA
LIVERMORE, CALIFORNIA 94550

TITLE: Mutagens from the cooking of foods.

PROJECT DIRECTOR: Frederick T. Hatch, MD

PROJECT OFFICER: Errol Zeiger, Ph.D., Supervisory Microbiologist

DATE AGREEMENT INITIATED: 1. September 22, 1978 (222-YO1-ES-80038)
2. April 1, 1981 (222-YO1-ES-10063)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this interagency agreement with the Department of Energy are to identify the mutagens produced in foods cooked under approximately normal household conditions and determine their mechanism(s) of formation, assess the spectrum of genetic toxicity caused by these mutagens using in vitro and in vivo short-term tests, devise strategies to limit or prevent mutagen formation and to estimate the normal dietary intake of these mutagens.

METHODS EMPLOYED: Hamburger is fried under normal cooking conditions, extracted, and the extracts tested for mutagenicity using the Salmonella plate test with S-9 preparations from mice, rats and hamsters pretreated with various inducers. Extracts exhibiting the highest levels of mutagenicity are separated in an attempt to isolate and identify the mutagenic components. Similar work is being done with other fried meats, fried eggs and beef extracts. Mutagenicity studies are being performed in Salmonella and in cultured CHO cells. Metabolism studies are being carried out using in vitro S-9 incubation conditions.

MAJOR FINDINGS AND PROPOSED COURSE: Hamburger: a series of extraction procedures have been developed which greatly increase the yield of extracted mutagen. The hamburger mutagens require metabolic activation and revert only those Salmonella strains which are reverted by frameshift mutagens. Studies on the kinetics of hamburger mutagen formation showed that the cooking temperature, rate of heat transfer and level of dehydration all affect the level of mutagenicity. Following a Japanese report which identified two imidazoquinolines (IQ and MeIQ) as beef mutagens, studies were performed, using preparative TLC followed by GC/MS and HPLC. A number of mutagenic fractions have been identified; the presence of IQ in one of the fractions has not been confirmed. Cold IQ and radiolabelled IQ have been synthesized and chemistry and mutagenicity studies have been initiated. Me-IQ will be synthesized in the near future. Extracts will be spiked with IQ and its location in the fractions pinpointed.

A number of metabolites of ^{3}H-IQ have been separated from an in vitro S-9 system. The metabolism of IQ is mediated by P-448. Only one of the metabolites - as yet undefined - is a direct mutagen for TA1538. Purification and identification of these matabolites is under way.

Cell culture mutagenesis. Trp-P-2 is a potent mutagen in mammalian cells (CHO), inducing gene mutations, SCE's, chromosome aberrations and micronuclei. IQ was weakly positive for these endpoints, and only in repair-deficient CHO cells. These results are in contradiction to the relative Salmonella results and studies are underway to resolve this problem. CHO cells will be exposed to the direct mutagen metabolite of IQ.

Boiled beef: Boiling beef to produce beef stock results in the formation of a product that is mutagenic to Salmonella in the presence of liver S-9. The highest level of mutation is found with extracts prepared at pH4 and pH9. Studies on proteolytic digests of beef extracts implied that soluble amino acids or polypeptides could influence the formation of mutagens.

Enhancement of mutagenic activity at pH4.0 is optimal after addition of tryptophan and creatinine PO_4 and results from reactions with components of less then 500 MW in the soluble portion of the beef extract. $FeSO_4$ addition further stimulated mutagen formation. The mutagens in boiled beef and Difco beef extract have been separated chromatographically and have also been reacted with nitrite. Results are consistent with the presence of at least two mutagenic components, one of which may be IQ.

Other foods: Extracts of fried eggs were also mutagenic, although the extraction and purification procedure that was optimal for fried beef was not optimal for fried egg. Studies are underway to identify the optimum extraction procedure for fried eggs and to determine why the mutagenic activity of egg extract appeared to be suppressed by the beef extraction procedure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
It is well accepted that mutagenic substances are normal components of the environment. In order to place human exposure levels of food mutagens in their proper perspective, it is important to characterize the levels and types of exposure and the biological activities of the mutagens. This project, which also includes a survey of food intake in the U.S., will attempt to produce estimates of this mutagen exposure. A major aspect of the program is the study of cooking practices or food additives (or treatments) which will retard or inhibit the formation of mutagens during the cooking process and thereby lower human exposure to these mutagens.

PUBLICATIONS

1. Felton, J., S. Healy, D. Stuermer, C. Berry, H. Timourian, F. Hatch, L. Bjeldanes, and M. Morris (1980). Improved Isolation and Characterization of Mutagenic Fractions from Cooked Ground Beef. Environ. Mutag. 2: 304.

1. UNIVERSITY OF WISCONSIN - Madison, Wisconsin 53707
(NO1-ES-9-0012)

2. BROWN UNIVERSITY - Providence, Rhode Island 02912
(NO1-ES-9-0015)

3. BOWLING GREEN STATE UNIVERSITY - Bowling Green, Ohio 43403
(NO1-ES-9-0016)

TITLE: Drosophila Mutagenesis Testing

CONTRACTOR'S PROJECT DIRECTOR: 1. Ruby Valencia, Ph.D. and Seymour Abrahamson, Ph.D.
2. Stanley Zimmering, Ph.D.
3. Ronald Woodruff, Ph.D.

PROJECT OFFICER: James Mason, Ph.D., Geneticist

DATE CONTRACT INITIATED: 1. September 28, 1979
2. September 28, 1979
3. September 28, 1979

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 60 environmental and commercial chemicals for mutagenicity using Drosophila melanogaster tester strains in three laboratories. Substances which are found to induce sex-linked recessive lethal mutations in Drosophila will be selected for testing in mammalian systems.

METHODS EMPLOYED: Standard sex-linked recessive lethal and reciprocal translocation tests in Drosophila melanogaster are being used to test for mutagenicity. Chemicals will be selected based on results obtained from previous mutagenicity tests using Salmonella. Chemicals will be administered by feeding and the sex-linked recessive lethal test will be performed. If the results are negative, the test will be repeated after injection. If the results are again negative, the chemical will be considered nonmutagenic in Drosophila. If the results are positive, the chemical will be tested in the reciprocal translocation test using the means of administration which gave the positive result. In the reciprocal translocation test sperm will be stored to enhance the ability to recover chromosome breaks induced by the chemicals. Results will be entered on data forms and transferred to a computerized data base system.

MAJOR FINDINGS AND PROPOSED COURSE: Results have been received from a total of 52 test samples to date which includes 47 unique chemicals. It is anticipated that an additional 40 samples will be tested this calendar year.

1. CASE WESTERN RESERVE UNIVERSITY - Cleveland, Ohio
(NO1-ES-9-2136)

2. MICROBIOLOGICAL ASSOCIATES - Rockville, Maryland
(EG&G MASON RESEARCH INSTITUTE)
(NO1-ES-9-2137)

3. SRI INTERNATIONAL - Menlo Park, California
(NO1-ES-9-0001)

TITLE: Microbial Mutagenesis Testing

CONTRACTOR'S PROJECT DIRECTORS: 1. William Speck, M.D.
2. Stephen Haworth, Ph.D.
3. Kristien Mortelmans, Ph.D.

PROJECT OFFICER: Errol Zeiger, Ph.D., Supervisory Microbiologist
Cellular and Genetic Toxicology Branch

DATE CONTRACTS INITIATED: 1. December 22, 1978
2. December 29, 1978
3. February 1, 1979

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test environmental and commercial chemicals for mutagenicity using Salmonella typhimurium tester strains in 3 laboratories. Based on results in Salmonella chemicals are selected for chemical analysis and further testing in Drosophila, for cytogenetic effects in cultured mammalian (Chinese Hamster ovary) cells, and for other genetic testing.

METHODS EMPLOYED: Salmonella typhimurium strains TA-98, TA-100, TA-1535, and TA-1537 are being used to test for mutagenicity using a modification of the Ames Salmonella microsome assay. All chemicals are incubated with tester strains in suspension prior to addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation is provided by liver S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats and Syrian Hamsters. All chemicals are tested blind at 5 doses, in triplicate, in each Salmonella strain. Also, all chemicals are retested at least one week following the first test. Results are being entered directly into a minicomputer for transfer to the data-base system.

MAJOR FINDINGS AND PROPOSED COURSE: Results have been received from these laboratories on a total of 692 test samples to date encompassing 545 unique chemicals. It is anticipated that an additional 400 samples will be completed this calendar year. These contracts will terminate in December, 1982 and January, 1983; competition is underway for replacement contracts.

In addition, numerous requests for information and data on specific chemicals tested have been received from government personnel and from the private sector. All information requested has been provided.

Results of these tests have been routinely published in the NTP Bulletin.

Manuscripts are currently being written to present results of the initial chemicals in reviewed scientific journals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
These contracts will allow the NTP to rapidly screen large numbers of chemicals for mutagenicity in a relatively short time and at relatively low cost. Mutagenicity in this system correlates strongly with carcinogenicity and heritable mutations in rodents. The results of these Salmonella tests will be used to assist in decisions regarding chemicals to be tested in sub-chronic and chronic toxicological tests.

1. ALLIED CORPORATION - Morristown, New Jersey
(NO1-CP-15764)

2. BIOASSAY SYSTEMS CORPORATION - Woburn, Massachusetts
(NO1-CP-15809)

TITLE: Development and Validation of a Multiple Endpoint Mutation System in Cultured Mammalian Cells.

CONTRACTOR'S PROJECT DIRECTORS: 1. J. Grant Brewen, Ph.D.
2. Kenneth Loveday, Ph.D.

PROJECT OFFICERS: Errol Zeiger, Ph.D., Supervisory Microbiologist
Robert Langenbach, Ph.D., Research Microbiologist
Cellular and Genetic Toxicology Branch

DATE CONTRACTS INITIATED: 1. September 30, 1981
2. September 30, 1981

PROJECT DESCRIPTION

OBJECTIVES: The objectives of these contracts are to develop, define and test a protocol (or series of protocols) using mammalian cells in culture to determine the frequencies of chemically-induced gene and chromosomal mutations. The possibility of determining other genetically-related endpoints such as sister chromatid exchange, DNA damage and repair, DNA adduct formation and aneuploidy are being considered. Once an acceptable protocol is developed, a number of coded chemcials will be tested.

METHODS EMPLOYED: Allied Corporation is investigating two cell lines, a human epithelial cell; HSBP, and CHO cells; Bioassay is using a CHO line. Both laboratories will standardize the culture and treatment conditions for each endpoint.

MAJOR FINDINGS AND PROPOSED COURSE: These contracts have recently been initiated and the majority of effort has been to define the optimum culture and treatment conditions for the different cell lines and to develop protocols for synchronizing the cells. Preliminary experiments have been run to determine the responses of the cells to a standard mutagen. These experiments will be continued and a number of mutagens will be used to induce gene mutations (at the HGPRT, and possibly the OUA locus), chromosome aberrations, sister chromatid exchanges and aneuploidy. Measurement of DNA damage and repair will also be made and Allied will also measure DNA-adduct formation. In addition the effects of liquid holding on the mutagenic and other responses will be determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The major types of effects of concern in genetic toxicology are gene and chromosome mutations. Genotoxic chemicals usually induce both types of

effects but the extent to which gene mutations or chromosome mutations is induced by any individual chemical is not predictable at this time. Gene and chromosome mutations are of interest because they both can produce human genetic disease.

Typically, induction of gene mutations in mammalian cells is detected in a number of different cell lines and the induction of chromosome mutations is usually detected using the same or different cell lines in laboratories specializing in cytogenetics. As a result, it is difficult to determine the relative frequencies induced and the effective doses. Yet, a comparison between gene and chromosome mutations as a function of chemical dose is needed as a reference when moving from results obtained with cells in culture to predicted effects in treated animals. Such an extrapolation is necessary when only one type of mutagenic effect can be measured *in vitro* but one wants to estimate the sum of both effects.

MICHIGAN CANCER FOUNDATION - Detroit, Michigan
(NO1-CP-15762)

TITLE: Modification of the Salmonella Test for Chemicals that may be Metabolized to Mutagens under Reductive Conditions.

CONTRACTOR'S PROJECT DIRECTOR: Charles King, Ph.D.

PROJECT OFFICER: Errol Zeiger, Ph.D., Supervisory Microbiologist
Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this contract are the development of Salmonella test protocols for detection of chemicals requiring reductive/anaerobic metabolism for their mutagenic activity, and the testing of chemicals for mutagenicity using these protocols. Among the chemicals tested will be a series of benzidine congenor dyes and urine samples from rats given benzidine dyes.

METHODS EMPLOYED: The contractor is investigating modifications of the Salmonella preincubation protocol which permit reductive metabolism followed by oxidative metabolism to measure to mutagenicity of benzidine-containing dyes. In addition, rat cecal flora preparations are being used in an attempt to develop an alternate activation system which is representative of the metabolism that occurs in the gut.

MAJOR FINDINGS AND PROPOSED COURSE: This project has recently been initiated and the majority of effort so far has been the definition of different metabolic activation systems and the investigations of chemical methods for purification of benzidine-based dyes. The sensitivity of various mutagenicity protocols to benzidine, dimethylbenzidine, dimethoxybenzidine and some model benzidine-based dyes are being evaluated. Also, the sensitivity of detection of benzidine metabolites in rat urine is being determined in preparation for a survey of urines from rats administered benzidine dyes. After the protocols have been standardized, a number of benzidine-congenor dyes will be tested for mutagenicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The standard protocols using in vitro metabolic activation for mutagenesis studies assume that the substances to be tested require aerobic metabolism for their activation. However, many substances, such as azo-containing dyes (including benzidine dyes), may be metabolized only by reductive pathways. These pathways occur in the mammalian liver in situ and in the mammalian gut through the action of the normal gut flora. Therefore, azo-containing chemicals which may be metabolized to mutagens in vivo may appear to be non-mutagenic when tested using the stardard metabolic (aerobic) activation protocols.

1. COLUMBIA UNIVERSITY - New York, New York
(NO1-ES-9-0014)

2. LITTON BIONETICS, INC. - Kensington, Maryland
(NO1-ES-9-0013)

TITLE: In Vitro Cytogenetic Testing

CONTRACTOR'S PROJECT DIRECTOR: 1. Arthur Bloom, M.D.
2. Sheila Galloway, Ph.D.

PROJECT OFFICERS: Errol Zeiger, Ph.D., Supervisory Microbiologist
Michael A. Resnick, Ph.D., Research Geneticist
Kenneth A. Palmer, Ph.D., Research Geneticist,
Division of Toxicology, FDA

DATE CONTRACT INITIATED: 1. September 29, 1979
2. September 29, 1979

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to develop and validate a protocol for testing a total of 350 chemicals for their ability to induce chromosome aberrations and sister chromatid exchanges in cultured Chinese Hamster ovary cells. In order to do this, the contractors are required to standardize and validate a protocol.

METHODS EMPLOYED: Chinese Hamster ovary cells in culture are being used to test for the induction of chromosome aberrations and sister chromatid exchange in vitro, both with and without S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats. The protocol has been developed and validated by the test laboratories. Results obtained from testing the unknown substances will be entered on standardized data forms and transferred to a computerized data base management system.

MAJOR FINDINGS AND PROPOSED COURSE: Seventy samples (including 52 unique chemicals) have been tested with and without S-9 activation in blind studies. The accumulated data has enabled the development of rigorous and statistically sound criteria for decision-making regarding evaluations of chemical responses. Nearly all chemicals which are positive in chromosome aberration tests are also positive in the sister chromatid exchange tests. Several chemicals which have been shown to be negative in the Salmonella tests have proven positive in the cytogenetics assays.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Salmonella test system currently in use is designed to detect substances which induce point mutations. It is not capable of detecting substances that produce only chromosome mutations. The Chinese Hamster ovary system will allow detection of chemicals which do not produce point mutations in Salmonella but are capable of producing chromosome aberrations in cultured mammalian cells. Sister chromatid exchange is being

used as an additional indicator for substances that are capable of damaging mammalian chromosomes. Chemicals found mutagenic in this system, regardless of their responses in Salmonella, will be given priority for chronic toxicological and carcinogenesis testing by the National Toxicology Program.

BROWN UNIVERSITY - Providence, Rhode Island 02912
(NO1-ES-1-5002)

TITLE: Development of a Drosophila Aneuploidy Test

CONTRACTOR'S PROJECT DIRECTOR: Stanley Zimmering, Ph.D.

PROJECT OFFICER (NIEHS): James M. Mason, Ph.D., Geneticist

DATE CONTRACT INITIATED: March 1, 1981

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this work is to develop a test system in Drosophila for screening environmental chemicals for their ability to induce aneuploidy. The use of a test for aneuploidy will allow us to identify chemicals which induce certain types of chromosomal aberrations which would not be identified as mutagenic in the standard short term mutagenesis test systems now is use.

METHODS EMPLOYED: The current project consists of two parts. During the first 18 months methods are being developed that will allow Drosophila to be used to test for chemically induced aneuploidy. The questions that are to be answered during this portion of the project are: (1) what endpoints will be scored; (2) the gender of the animal to be tested; (3) the developmental stage to be treated; (4) means of administration; and (5) appropriate sample size. During the second 18 months, a standard protocol based on these findings will be tested using coded control chemicals.

MAJOR FINDINGS AND PROPOSED COURSE: Most of the above issues have been settled. (1) Low level effects are best scored by monitoring segregation of X from Y. This test should be more sensitive to disruptions in recombination or distributive pairings than is X from X segregation, although in the absence of chemicals known to disrupt these processes, this cannot be tested directly. Strong effects can best be seen by scoring toward the presence of triploids. These two endpoints can be monitored in the same test, which greatly facilitates screening. (2) The gender seems to have little bearing on the effectiveness of a chemical. Therefore, females will be used because meiosis in females is very similar to meiosis in other organisms. (3 & 4) Larvae will be fed chemicals because of the ease of administration and because this will allow mitotic as well as meiotic stages to be treated. (5) A sample size remains to be determined. This will depend on the background sequencies of the endpoints. More data are needed to determine these more precisely. Large sample sizes can easily be screened for two reasons: (1) a single generation test can be used and (2) flies can be weighed instead of counted. This procedure has been shown to be accurate to within 1%.

A protocol is now being written based on these findings. As soon as this protocol is approved, testing will begin on coded control chemicals. The first chemicals to be tested will include: a mono-functional alkylating agent, long chain fatty acids and inhibitors of energy metabolism. Preliminary tests with uncoded chemicals have been made. Colchicine is strongly positive, inducing a response at 5μg/ml. Caffeine was negative and TWEEN 80 was weakly positive.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
This project is designed to develop a test system to be used as part of the Cellular and Genetic Toxicology Branch Mutagen Screening Program. The use of a test for aneuploidy will allow us to identify chemicals which induce certain types of chromosomal aberrations and would not be identified as mutagenic in other mutagenesis test systems. A large fraction of spontaneous abortion in humans and certain serious genetic diseases (e.g., Down's syndrome) are caused by aneuploidy. A few chemicals are known to induce aneuploidy; however, there is no fast, reliable, well-defined and developed test to detect such chemicals on a large scale.

OAK RIDGE NATIONAL LABORATORIES, Oak Ridge, Tennessee 37830

(222Y01-ES-80041)

TITLE: Study of Antigenic Markers in Developing Epithelial Neoplasia

CONTRACTOR'S PROJECT DIRECTOR: S. J. Kennel, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

DATE CONTRACT INITIATED: October 1, 1980

CURRENT ANNUAL LEVEL: $135,000

PROJECT DESCRIPTION

OBJECTIVES: This study involves changes in phenotypic expression of antigenic markers on tracheal epithelial cells as they progress from preneoplastic (non-tumorigenic) to neoplastic (tumorigenic) cell populations in tissue culture. Carcinogen-altered tracheal cell lines have been established from carcinogen exposed F344 rat tracheas. Many of these continuous cell lines are not capable of forming tumors when inoculated into compatible animals, but acquire this ability upon serial passage in vitro. These carcinogen-altered cell lines are phenotypically distinguishable from normal populations by the appearance of new cell surface antigens (Braslawsky et al., 1981, 1982a, 1982c; Nettesheim, 1982) and altered DNA content (Braslawsky et al., 1982b, 1982c). The principal objective of this research project is to investigate changes in phenotypic expression of carcinogen-altered cell lines as preneoplastic cultures progress from non-tumorigenic to tumorigenic populations, and to correlate changes in tumor antigen expression and DNA content with malignant transformation in vitro.

METHODS EMPLOYED: Monoclonal antibodies were prepared to tumorigenic cell lines. Donor spleen cells from transplantation resistant rats were fused with the mouse myeloma P3-X63-Ag8 and donor spleen cells from immunized BALB/c mice were fused to the mouse myeloma SP2/0 (Kennel et al., 1981). Serological recognition of cell surface antigens are divided into two categories: Those which assess average antigen expression of the cell population and those which measure antigen expression and density of individual cells within the population. The radio-labeled antibody binding test is of the first type. This test can quantitate the amount of tumor antigen on preneoplastic and neoplastic cell populations. The fluorescent antibody test combined with flow-cytometry is of the second type, which analyzes cell populations for fluorescent yield per cell as well as enumerates the percentage of antigen positive cells. Relative DNA content is determined by incorporation of DNA specific fluorescent dyes and quantitated by flow cytometric methods.

MAJOR FINDINGS AND PROPOSED COURSE: At least two major classes of tumor antigens that do not appear on normal tracheal epithelial cells have been identified using F344 rats immunized to the neoplastic phase cell lines (Braslawsky et al., 1981). The antiserum response has been characterized for their specificity of

of reaction and titer, but could not distinguish preneoplastic from neoplastic populations either qualitatively or quantitatively (Braslawsky et al., 1982a). These populations can be morphologically distinguished on the basis of a change in relative DNA content that occurs as preneoplastic populations become neoplastic in vitro (Braslawsky et al., 1982b, 1982c; Nettesheim et al., 1982).

The complexity of the syngeneic response in defining antigens appearing on malignant phase cells has prompted us to produce monospecific antibodies to tracheal tumor antigens. Fifteen monoclonal antibodies have been isolated and eleven identified that react with tracheal carcinoma cells, but not normal populations (Braslawsky et al., 1982d). These monoclonal antibodies have been tested by the ABT on preneoplastic and neoplastic phase of 5 respiratory tract cell lines, and three of them could distinguish preneoplastic populations from neoplastic populations on the basis of increased binding activity (Braslawsky et al., 1982d).

The coordinate changes in DNA content and antigen expression defined by monoclonal antibodies as cultures shift from non-tumorigenic to tumorigenic populations is now under study. The results indicate that both altered DNA contents and antigen expression appear simultaneously as transformed tracheal epithelial cells adapt to culture and form non-tumorigenic populations, and change as the cultures become tumorigenic with no detectable intermediate phenotypes. Simultaneous detection of altered DNA and antigen content by flow cytometry is possible if sensitive assays for cell surface antigens can be developed using FITC-labeled reagents. Such assays would have significantly increased sensitivity and allow for isolation and quantitation of altered cells soon after chemical treatment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The appearance of tumor markers which can be recognized immunologically during the period when cells have altered (transformed) phenotypes, but lack oncogenic ability (malignant neoplasia), would be invaluable in understanding the oncogenic process. These studies will provide information on the appearance of tumor antigens after carcinogenic insult and the frequency of antigen positive cells in exposed populations before the appearance of neoplastic cell populations. The isolation of antigen-positive phenotypes in mixed populations will also allow study of progenitor-progeny relationships that have evolved as a result of carcinogenesis initiation. This will enable us to determine if antigen positive cells have a selective growth advantage over non-antigenic bearing cells and whether the quantity of antigen expressed, changes as a function of neoplastic differentiation in vitro. Assay systems used in these experimental animal model systems in the future will provide the basis for defining human respiratory tract tumor markers and may also be useful for short term testing of malignant transformation potential of putative carcinogens.

Braslawsky, G.R., Steele, V., Kennek. S.J., and Nettesheim, P.: Syngeneic immune response to rat tracheal epithelial cells transformed in vitro by N-methyl-N'-nitro-N-nitrosoguanidine. Brit. J. Cancer 44, 247-257, 1981.

Braslawsky, G.R., Kennel, S.J., Steele, V., and Nettesheim, P.: Detection of tumor gantigens by syngeneic antiserum on in vitro transformed tracheal epithelial cell line 2-10-1. Int. J. Cancer 29(6), in press, 1982a.

Nettesheim, P., Braslawsky, G.R., Steele, V., and Kennel, S.J.: Cell populations studies during epithelial carcinogenesis. In: Tumor Cell Heterogeneity: Origins and Implications. Vol. 4, Ed. Owens, Academic Press, Inc., New York, 1982.

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Kennel, S.J., Foote, L.J., and Lankford, P.K.: Analysis of surface proteins of mouse lung carcinomas using monoclonal antibodes. Cancer Res. 41, 3465-3470, 198o.

Braslawsky, G.R., Flynn, K., Steele, V. and Nettesheim, P.: Changes in DNA content of rat tracheal epithelial cells curing neoplastic progression in vitro. Carcinogenesis, submitted, 1982c.

CHEMICAL PATHOLOGY BRANCH

CHEMICAL PATHOLOGY BRANCH

Summary Statement

Mission: During FY 81 the Chemical Pathology Branch continued its three major functions: (1) support of the National Toxicology Program, [40%]; (2) support of Intramural Research [35%]; and (3) independent research [25%].

Section Work Areas: Histology and Electron Microscopy, Tumor Pathology, Toxicologic Pathology and Experimental Pathology.

Staffing: The Chemical Pathology Branch consists of 7 comparative pathologists, 10 technicians, 3 secretaries and 2 stay-in-schoolers. Dr. Scot Eustis, formerly of South Dakota State University joined the staff in June 1982. He has been assigned to the Tumor Pathology Section. Dr. Carolyn Lingeman joined the Branch in April 1982. She will be assisting the Branch in the review of NTP Technical Reports and will work on specific projects dealing with acute and chronic bioassays.

Accomplishments:

1. Management of Quality Assurance Program for the National Toxicology Program - During FY82 the Chemical Pathology Branch continued responsibility for evaluating the quality of pathology conducted in bioassays performed by the NTP. This included the review of 30 prechronic and 27 chronic bioassays during the first 9 months of FY82 (Tables 1 and 2).

2. Implementation of TDMS - During FY82 the Toxicology Data Management System (TDMS) was implemented in two contractor laboratories. Emphasis by the Branch was placed on completing the micropathology glossary and development of the gross pathology glossary.

3. Research Programs - Studies in support of the National Toxicology Program included:

   a. revision of the pathology portion of the life-time carcinogen bioassay protocol for the purpose of reducing the volume of pathology

   b. defining of criteria to be used for the diagnosis of proliferative lesions of the pituitary, thyroid, adrenal, pancreas and lung

   c. investigations on the relationship of specific tumor types to cause of death

   d. monitoring of the pathology aspects of the oral (ingestion) asbestos studies in rats being conducted at Hazleton Laboratories

   e. evaluation of lesions produced by polybrominated biphenyls in rats and mice following a 6 month exposure with subsequent life time observation

   f. evaluation of the comparative toxicity of C.I. Direct Blue 6 and benzidine in rats

g. evaluation of lesions produced by inhalation of various forms of asbestos and glass wool in rats; this study is being conducted in conjunction with Dr. Wagner, Pneumoconiosis Research Unit, Medical Research Council, England

h. evaluation of the Strain A lung adenoma model to evaluate the carcinogenic potential of chemicals

i. studies on the pathogenesis of hepatotoxicity caused by furfural alcohol

j. ultrastructural studies of lung lesions induced by 2,3-dibromo-1-propanol.

4. Research Program - Independent studies and collaborative efforts with other laboratories in TRTP/NTP:

a. immunotoxic and myelotoxic effects of twelve different chemicals

b. validate and develop clinical chemistry methods for use in pathology and toxicity methods

c. toxic effects of allyl isothiocyanate

d. toxic effects of 8-methoxypsoralen

e. evaluation of the effects of kepone on male reproductive capacity.

5. Support of the Intramural Research Program - A great deal of support was provided in support of the Laboratory of Reproductive and Developmental Toxicology, Laboratory of Organ Function and Toxicology, Laboratory of Environmental Chemistry, and Laboratory of Pulmonary Function and Toxicology. Lesser support was provided to Laboratory of Biochemical Genetics, Laboratory of Molecular Genetics, Laboratory of Behavioral and Neurological Toxicology and Laboratory of Pharmacology.

a. Laboratory of Reproductive and Developmental Toxicology

(1) Dr. J. McLachlan - consultations on lesions found in mice exposed *in utero* to diethylstilbestrol (DES) and related compounds

(2) Dr. F.R. Sim - effect of jervine on rat embryos *in vitro*

(3) Dr. J. McLachlan - electron microscopy on CD-1 mouse fetal genital tract culture after prenatal treatment with DES

(4) Drs. Bachter, Weber, Ettlin and Dixon - effects of anti-cancer drugs on the immature testes

(5) Dr. J. McLachlan - electron microscopy and special histologic techniques in the study of the effects of DES on the developing and adult reproductive tract.

b. Laboratory of Pharmacology

(1) Drs. C. Schiller and C. Shoaf - effects of TCDD on gut, liver, pancreas and lungs of rats

(2) Dr. B. Fowler - several projects involving EM support of studies on the ultrastructural effects of heavy metals on the kidney and liver

(3) Dr. K. Jones - drug and xenobiotic metabolism in the lungs; mechanisms and modifying factors

(4) Drs. J. Fouts, M. Coomes and Ms. R. Pohl - xenobiotic - metabolizing enzyme activity in skin and its response to environmental agents

c. Laboratory of Pulmonary Function and Toxicology

(1) Dr. R. DiAugustine - neuroendocrine epithelial cells of the guinea pig upper respiratory tract

(2) Dr. Hook - studies on the composition and ultrastructure of abnormal tubular myelin assembly in the lungs of patients with pulmonary alveolar proteinosis

(3) Dr. A. Brody - deposition and translocation of inhaled asbestos

(4) Drs. C. Barrett and E. Sisskin - ultrastructure of cultured epidermal cells as a model for skin carcinogenesis

(5) Dr. K. Sonstegard - neuroendocrine cells in rabbit fetal lung as a model for in-depth study

(6) Dr. A. Brody - deposition and translocation of tracheal crystalline silica

(7) Dr. R. Wu - serial cultivation of tracheal epithelial cells in vitro

(8) Dr. R. Wu - characterization of primary tracheal epithelial cells in culture.

Publications (Branch Personnel Underlined)

a. Book Chapters and Articles

(1) Abdo, K., Haseman, J.K., Farnell, D., Prejean, J.D., Boorman, G.A. and Kovatch, R.: Absence of carcinogenic response in F344 rats and B6C3F1 mice after feeding d-mannitol in the diet for two years. Food Cosmet. Toxicol. (submitted) (1982).

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(4) Boorman, G.A.: Follicular cell hyperplasia, adenoma and carcinoma in rats. In: Tumor of Rat Thyroid. ILSI Monograph on Pathology of Laboratory Animals (in press) (1982).

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b. Abstracts

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TABLE 1

Chronic Bioassays Reviewed from October 1981 to June 1982

Chemical	Species
Allyl Isovalerate (C54717)	Rats/Mice
Amosite (C60253)	Rats
C.I. Acid Yellow (Fluorescein Disodium Salt) (C54706)	Rats/Mice
Cyclohexanone (C55005)	Rats
Cyclohexanone (C55005)	Mice
1,2-Dichloropropane (C55141)	Rats/Mice
Diglycidyl Resorcinol Ether (C54996)	Rats/Mice
DMBA-TPA (C03985A)	Rats
Dodecyl Alcohol (Ethoxylated) (C54875)	Rats/Mice
Ethyl Acrylate (C50384)	Rats/Mice
Methylene Chloride (C50102)	Rats
Methylene Chloride (C50102)	Mice
Methylene Chloride (C50102)	Rats (Re-evaluation)
Monochlorobenzene (C54886)	Rats/Mice
Orthodichlorobenzene (C54944)	Rats/Mice
Sodium (2 Ethyl Hexyl) Alcohol Sulfate	Rats/Mice
Sodium Dodecyl Sulfate (C50191)	Rats/Mice
SR Chrysotile (C61223)	Rats
Tetrachloroethylene (C04580)	Mice

Chemical	Species
Tetrachloroethylene (C04580)	Rats (Long-Evans)
Tetrachloroethylene (C04580)	Rats
Tetrachloroethylene (C04580)	Rats (Wistar)
Tremolite (C08991)	Rats
Trichloroethylene (C04546)	Rats
1,1,1-Trichloroethane (C04626)	Rats/Mice
2,4 Toluene Diisocyanate (C50533)	Rats/Mice
2,6 Xylidine	Rats

TABLE 2

Pathology Evaluation on Immunotoxicology Studies

Phenobarbitol	Neonatal Phorbol Ester
Ochratoxin	Urethane
Mercury Chloride	Polyvinyl Pyrrolidone
Asbestos	Estradiol
Estradiol Plus Progesterone	Estradiol Metabolites
Zearalenone	Promethazine

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES21007-02 CPB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Chronic Exposure to Airborne Environmental Agents - Particulates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	STB NIEHS
OTHER	E.E. McConnell	Veterinary Pathologist	CPB NIEHS
	G.A. Boorman	Veterinary Pathologist	CPB NIEHS
	J.K. Haseman	Statistician	BRAP NIEHS

COOPERATING UNITS (if any)

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LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various forms of chrysotile asbestos (short range, intermediate range, and Canadian Standard B) and glass microfiber (JM-100) were given to male and female rats in an inhalation chamber 7 hours/day, 5 days/week for 12 months. The rats were then held for life time observation. Groups of animals were killed at 3 months and 12 months exposure and 12 months postexposure to evaluate lung lesions. All forms of asbestos (especially the longer fibers) caused fibrosis in the area of the terminal bronchioles which progressed during exposure but remained relatively static during the postexposure period. The glass wool did not produce this lesion. Treatment related pulmonary carcinomas were observed in rats exposed to asbestos, with more being observed with the intermediate range and chrysotile B than with the short range. No treatment related neoplasms were observed with JM-100.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The fiber (asbestos and glass wool) studies were conducted using male and female Fischer 344 rats which were exposed for 7 hr/day, 5 days/week for 12 months. The animals will then be held for their life-time. The types of asbestos used were short range (<5μm in length) chrysotile, chrysotile B, and NIEHS intermediate; the glass microfiber was Johns-Manville 100. The object of the study was to determine and compare the fiber retention in the lung and fibrogenic and carcinogenic potential of these fibers using standard physiological and pathology techniques. An additional morphometric study using electron microscopy is being conducted to study and quantitate the early changes in the lung produced by these fibers.

MAJOR FINDINGS AND PROPOSED COURSE: All of the rats on the life time portion of the study have died. Interim sacrifices at 3, 12, and 24 months (12 months post-exposure) were also conducted. Pathology results from the interim animals showed asbestosis (pulmonary fibrosis) which was progressive during the exposure phase and remained relatively static during the 12 month postexposure phase. Asbestosis was more severe with the intermediate range chrysotile and chrysotile B than the short range chrysotile (Table 1). The glass fibers caused only minimal changes in the lung. Results from the lifetime observations showed that all forms of asbestos caused pulmonary carcinomas, with fewer being observed with short range chrysotile (Table 2). The glass microfiber did not cause neoplasia. This study was completed in FY 82.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information gained from the asbestos studies will have immediate societal impact since the problems associated with the inhalation of asbestos in humans are well known. One of the major forms of insulation presently being used is glass wool, therefore the information gained from this aspect of the study is extremely important.

PUBLICATIONS

McConnell, E.E., Wagner, J.C., Moore, J.A., and Berry, G.: Comparable effects of inhalation USA/UK. Proceedings of Conference on Biological Effects of Man-Made Mineral Fibers-Occupational Health Conference-WHO/EURO. Copenhagen, 1982, in press.

TABLE 1

Severity of Pulmonary Fibrosis in Male F344 Rats Exposed to UICC Chrysotile B Asbestos or Glass Microfiber (JM-100)

Fiber Type	Age	Degree of Fibrosis-Grade NIEHS	MRC
Control	3	(10) 1.0	(8) 1.0
UICC Chrysotile	3	(6) 2.3	(8) 3.0
Glass Fiber	3	(6) 2.1	(8) 2.8
Control	12	(8) 1.2	(6) 1.0
UICC Chrysotile	12	(8) 3.3	(6) 4.0
Glass Fiber	12	(8) 2.0	(6) 2.8
Control	24	(8) 1.3	(6) 1.0
UICC Chrysotile	24	(8) 3.6	(6) 4.5
Glass Fiber	24	(8) 2.1	(6) 3.5

( ) - Number of animals. Males and females combined.

TABLE 2

Incidence of Pulmonary Neoplasia[a] in F344 Rats Exposed to UICC Chrysotile B Asbestos or Glass Microfiber (JM-100)

Fiber Type	Sex	No.	Bronchoalveolar Hyperplasia	Adenoma	Adenocarcinomas	Total Tumors
Control/MRC	M	24	1	0	0	0
	F	24	0	0	0	0
Control/NIEHS	M	27	0	1	2	3
	F	26	0	0	0	0
UICC Chrysotile/MRC	M	24	3	1	6	7
	F	24	2	0	5	5
UICC Chrysotile/ NIEHS	M	29	2	3	6	9
	F	27	3	1	1	2
Glass Fiber/MRC	M	24	3	0	1	1
	F	24	0	0	0	0
Glass Fiber/NIEHS	M	28	0	0	0	0
	F	27	0	0	0	0

[a]Only the most severe lesion appears in this table, e.g. if a rat had an adenoma, then bronchoalveolar hyperplasia would not be included.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES21008-02 CPB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Carcinogenic Potential of Polybrominated Biphenyl Mixture in the Rat and Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	STB NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	CPB NIEHS
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	D.L. Myers	Biological Laboratory Technician	CPB NIEHS
	R.E. Wilson	Biological Laboratory Technician	CPB NIEHS
	J.A. Haseman	Statistician	BRAP NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH
Chemical Pathology Branch

SECTION
Toxicologic Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polybrominated biphenyl mixture (Firemaster FF-1) was given orally to rats and mice at 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg body weight, 125 total doses over a 6-month period. After the 6-month exposure to PBB, the animals were held for an additional 2-year period for lifetime observation.

Polybrominated biphenyl mixture (Firemaster FF-1) was carcinogenic for Fischer 344 rats and B6C3F1 mice of both sexes. The higher incidence of hepatic neoplasms included neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas in rats and hepatocellular carcinomas in mice. Other toxicities included porphyrogenic effects, hepatotoxicity, chronic progressive nephropathy and hyperplastic gastropathy in the rat. The PBB mixture also affected the body weight gain in male and female rats and male mice although there was no significant difference in food consumption.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Polybrominated biphenyl mixture, Lot No. 1312FT, Batch 03, was given to rats (Fischer 344) and mice (B6C3F1) via gavage (125 total doses, 5 days/week) for a period of 6 months at 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg body weight. After termination of 6-month exposure, rats and mice (treated and control of both sexes) were kept for 23 and 24 additional months respectively for life-time observation.

MAJOR FINDINGS AND PROPOSED COURSE: The treatment (1.0 mg or higher dose levels) shortened the survival time in male rats. A significantly high incidence of atypical hepatocellular foci, neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas were observed in exposed rats (Tables 1 and 2). The incidence of hepatocellular carcinomas was also increased in both male (95%) and female (88%) mice (highest dose level) compared with control male (48%) and female (0%) mice (Table 3). The incidence of hepatic neoplasms appeared to be dose dependent in rats and mice. Liver tumors were observed only in those groups of animals to which PBB was given in doses sufficient to induce readily observable hepatic toxicity. This study was completed in FY 82.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fire retardant, Firemaster FF-1, a mixture of polybrominated biphenyls, was responsible for widespread environmental contamination, animal loss and possibly human illness in Michigan during 1973-1974. Long term effects in animals and possibly humans are still apparent because of the extremely long biological half-life and persistance in the environment. Because of this, it is important to evaluate in depth the long term toxicologic effects of the compound in laboratory animals in order to project the possible effects in man.

PUBLICATIONS

Gupta, B.N., McConnell, E.E., Harris, M.W., and Moore, J.A.: Polybrominated biphenyl toxicosis in the rat and mouse. Toxicol. Appl. Pharmacol. 57: 99-118, 1981.

Gupta, B.N., McConnell, E.E., Haseman, J.K., Harris, M.W., and Moore, J.A.: NTP technical report on the toxicology and carcinogenesis bioassay of polybrominated biphenyl mixture (Firemaster FF-1). NTP-81-26, in press.

Gupta, B.N., McConnell, E.E., Harris, M.W., and Moore, J.A.: Six-month exposure of a polybrominated biphenyl mixture in the rat and mouse. Toxicol. Appl. Pharmacol., in press.

Gupta, B.N., McConnell, E.E., Haseman, J.K., and Moore, J.A.: Carcinogenic potential of a polybrominated biphenyl mixture in the rat and mouse. Toxicol. Appl. Pharmacol., in press.

TABLE 1

Incidence (%) of Microscopic Lesions in the Liver of Male Rats After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Dose mg/kg	Atypical/Foci	Neoplastic Nodules	Hepatocellular Carcinoma	Bile Duct Hyperplasia	Cholangio-carcinoma
0	3(1/33) *	0(0/33)	0(0/33)	24(8/33)	0(0/33)
0.1	8(8/39)	0(0/39)	5(2/39)	23(9/39)	0(0/39)
0.3	30(12/40)[b]	2(1/40)[a]	0(0/40)	25(10/40)	0(0/40)
1.0	35(11/31)[b]	13(4/31)[a]	3(1/33)	42(13/31)	0(0/31)
3.0	39(13/33)[b]	12(4/33)	21(7/33)[b]	42(14/33)	0(0/33)
10.0	39(12/31)[b]	3(1/31)	23(7/31)[b]	29(9/31)	6(2/31)[a]
Dose Response	P<.01	NS	P<.01	NS	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.05) increased compared with control.
b Significantly (P<.01) increased compared with control.

TABLE 2

Incidence (%) of Microscopic Lesions in the Liver of Female Rats After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Dose mg/kg	Atypical/Foci	Neoplastic Nodules	Hepatocellular Carcinoma	Bile Duct Hyperplasia	Cholangio-carcinoma
0	0(0/20) *	0(0/20)	0(0/20)	10(2/20)	0(0/20)
0.1	0(0/21)	10(2/21)	0(0/21)	0(0/21)	0(0/21)
0.3	5(1/21)	0(0/21)	0(0/21)	0(0/21)	0(0/21)
1.0	18(2/11)	18(2/11)[a]	0(0/11)	9(1/11)	0(0/11)
3.0	21(4/19)	26(5/19)[a]	16(3/19)[a]	21(4/19)	0(0/19)
10.0	40(8/20)[a]	40(8/20)[a]	35(7/20)[a]	35(7/20)	35(7/20)[a]
Dose Response	P<.01	P<.01	P<.01	P<.01	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.01) increased compared with control.

TABLE 3

Incidence (%) of Microscopic Lesions in the Liver of Male and Female Mice After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Sex	Dose (mg/kg)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Metastasis to Lung
Male	0	8(2/25)*	48(12/25)	16(4/25)
	0.1	4(1/27)	30(8/27)	0(0/27)
	0.3	17(4/24)	33(8/24)	8(2/24)
	1.0	8(2/25)	48(12/25)	8(2/25)
	3.0	9(2/23)	65(15/23)	13(3/23)
	10.0	5(1/22)	95(21/22)[b]	18(4/22)
	Dose Response	NS	P<.01	P=.03
Female	0	0(0/13)	0(0/13)	0(0/19)
	0.1	11(2/19)	0(0/19)	0(0/19)
	0.3	0(0/15)	13(2/15)	0(0/15)
	1.0	9(1/11)	18(2/11)	9(1/11)
	3.0	6(1/17)	18(3/17)	6(1/17)
	10.0	12(1/8)	88(7/8)[b]	38(3/8)[a]
	Dose Response	NS	P<.01	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.05) increased compared with controls.
b Significantly (P<.01) increased compared with controls.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES21006-02 CPB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Chronic Exposure to Airborne Environmental Agents - Vinyl Chloride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	STB NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	CPB NIEHS
	G.A. Boorman	Veterinary Pathologist	CPB NIEHS
	J.K. Haseman	Statistician	BRAP NIEHS

COOPERATING UNITS (if any)

Becton Dickinson & Co., Research Triangle Park, North Carolina 27709
Experimental Pathology Laboratory, Raleigh, North Carolina 27606

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vinyl chloride (VC) was administered via inhalation to groups of female hamsters, rats, and 2 strains of mice using various dosing regimens in an effort to compare the carcinogenic response. The different groups were exposed to VC for 6 hours/day, 5 days/week as follows: 0-6 months, 12-18 months, 12-24 months and 18-24 months following the start (animals were 8 weeks of age at start) of the experiment.

VC related tumors were dependent on both the age of the animal during exposure and duration of exposure. In general, the younger the animal when exposed the higher the incidence of tumors when compared to groups exposed for the same length of time but starting with older animals. Exposures of longer than 12 months did not significantly increase tumor incidence.

PROJECT DESCRIPTION

METHODS EMPLOYED: The vinyl chloride study was designed to compare the carcinogenic response in female hamsters, rats and two strains of mice to various dosing regimens. The hamsters were exposed to 200 ppm VC, mice to 50 ppm VC and the rats to 100 ppm VC; levels known to be carcinogenic for each species. The different groups were exposed as follows: 0-6 months, 0-12 months, 0-18 months, life-time (24 months), 6-12 months, 6-18 months, 12-18 months, 12-24 months, and 18-24 months following the start of the experiment. The animals were 2 months of age at the beginning of the experiment.

MAJOR FINDINGS AND PROPOSED COURSE: In the vinyl chloride studies the rat and hamster tissues have been examined and the results tabulated. In rats there is a dose response increase in angiosarcomas with increasing exposure durations. Rats exposed to 0, 0-6, 0-12, 0-18, and 0-24 months having 2, 5, 21, 26 and 42% angiosarcomas respectively. Exposures at 12-18 and 12-24 months did not result in a significant increase in angiosarcomas, suggesting age at time of exposure is a critical factor. Hepatocellular carcinomas and mammary gland carcinomas were also increased following vinyl chloride exposure. In hamsters, the highest incidence (15%) of angiosarcomas was found in hamsters exposed 0-6 months to vinyl chloride. Increasing exposure duration or beginning later in life results in the production of a lower incidence of angiosarcomas. Mammary gland carcinomas were also increased in hamsters following vinyl chloride exposure, however, 0-6 months exposure appears as effective in producing tumors as does longer exposure regimens. VC was also carcinogenic in both strains of mice, causing similar types of tumors. In both strains the highest incidence of malignant tumors occurred in animals exposed in the first 6 months of the study. The results are completed and a manuscript submitted for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The objective of the vinyl chloride study was to evaluate various exposure regimens using a known carcinogen. With that knowledge gained from this study it is hoped that in the future inhalation studies can be better designed in an effort to better utilize the limited resources available to inhalation toxicologists. For instance, this information might allow shorter exposures, thereby significantly reducing the expense of such studies.

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. - Herndon, Virginia 22070

NO1-CP-95618

TITLE: Pathology Support for the Carcinogenesis Testing Program (Task I)

CONTRACTOR'S PROJECT DIRECTOR: Dr. J.F. Hardisty and Dr. W.O. Iverson

PROJECT OFFICER (NIEHS): Dr. E.E. McConnell, Chief, Chemical Pathology Branch

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: To provide (1) the necessary professional and technical personnel and facilities to process tissues for light and electron microscopy; (2) perform the gross and/or histopathologic evaluation on animal tissues generated within the National Toxicology Program (NTP); (3) conduct electron microscopic evaluation of animal tissues; (4) participate in advisory groups, workshops, seminars, and site visits; and (5) provide training in gross necropsy and histologic techniques.

METHODS EMPLOYED: The above objectives are carried out by use of standard histopathologic methods and equipment. They include those items commonly found in histology and pathology laboratories.

MAJOR FINDINGS AND PROPOSED COURSE:

A. The contractor was requested to provide the pathologist and technical staff necessary to perform the necropsies of 240 rats at Litton Bionetics (LBI) in Kensington, Maryland. The rats represented the terminal sacrifice for the carcinogen bioassay of 2,6-Dimethylaniline conducted by EPA and NCI. The necropsies were performed from September 9-11, 1980. The histology laboratory of the contractor has processed the tissues from these animals and from 210 interim death rats. All necropsies and all tissue processing were conducted in accordance with the most recent NCI protocol. The contractor is currently performing the microscopic evaluation of the tissues from these animals and is completing a pathology report detailing the findings. Electron microscopy of three nasal tumors encountered in this study is currently being performed to confirm their identity.

B. The contractor obtained the formalin fixed heads of 392 rats from Midwest Research Institute in Kansas City, Missouri. These animals were from a study conducted by Midwest Research Institute on the toxicity of inhaled 1,2-Dibromoethane in rats with and without disulfiram in the diet as part of the National Toxicology Program and sponsored by NIOSH and NCI/NTP.

The histology laboratory of the contractor prepared and processed cross sections through the nasal cavities of these animals and sections of brain when invasive tumors were observed. Additionally, the contractor collected portions of twenty nasal tumors for ultrastructural studies. A pathologist from the contractor laboratory performed the microscopic evaluation of the

tissue taken for light microscopy and has submitted a pathology report detailing the findings and a slide set of the required tissues. Similarly, the contractor's electron microscopist/pathologist has characterized the ultrastructural features of the specimens obtained for EM in a report which has been submitted.

C. The contractor performed necropsies on a number of Sencar mice utilized in a diesel exhaust inhalation exposure study. During the course of the necropsies, additional specimens of untreated control mouse kidneys were obtained for light and electron microscopic evaluation. The histology laboratory of the contractor has completed the processing of H&E sections of these kidneys. The contractor's pathologist reviewed the sections and submitted them to Dr. Jerrold Ward for his evaluation. Based upon his findings, Dr. Ward selected three animals which were to have the kidneys examined by EM. The contractor has processed and prepared electron photomicrographs of these specimens. The contractor's microscopist/pathologist has submitted a report characterzing the ultrastructural features of these specimens. This effort is a continuation of a progressive study to define a spontaneous lesion observed in the Sencar mouse.

Additionally, the pathologist and technical staff of the contractor collected specimens of nineteen lung tumors observed in these mice at the time of necropsy. Hematoxylin and eosin stained slides have been prepared by the histology laboratory of the contractor and will be examined to determine the feasibility of performing EM on some of these tumors.

D. The contractor was requested to microscopically examine hematoxylin and eosin stained tissue sections from rats and hamsters used in a study of the carcinogenic potential of inhaled oxide of nitrogen in animals exposed to organic amines in their drinking water. These evaluations are being performed by a contractor pathologist and the report has been submitted.

E. The contractor was requested to grossly examine and record the lung tumors from 185 mice from the bioassay system studies (Chemicals #3452 and #5269) conducted by the University of San Diego, School of Medicine. All lung tumors were counted and processed in such a manner to allow correlation of the microscopic lesion to the gross observation. These tissues have been processed by the histology laboratory of the contractor. A contractor pathologist has examined the tissues and the report has been submitted.

F. The contractor was requested to perform the microscopic evaluation of the mice and rats from two subacute (90 day) studies conducted by Cannon Laboratories on Phenolphthalen and Oxalic Acid. Contractor pathologists have performed the histopathologic evaluation of the slides for these studies and the reports have been submitted.

G. The contractor was requested to process for EM evaluation, the formalin fixed liver tumor tissue from a B6C3F1 mouse from the bioassay of Gum Arabic. The EM laboratory of the contractor has completed the processing of the tissue submitted and electron photomicrographs of what appears to be an Ito-cell tumor were submitted to Dr. Ward.

H. Similarly, the contractor was requested to prepare, from paraffin blocks, specimens of several thymic tumors from Fischer 344 rats for EM evaluation. This project attempted to characterize the ultrastructural features of several of these thymic tumors. The paraffin embedded tissue was of poor quality for electron microscopy, but electron micrographs from several representative specimens have been submitted.

I. The contractor was requested to make frozen sections and subsequently prepare fat stains on samples of liver tissue from five rats which were part of the bioassay of Allyl Isothiocyanate. The request for this support was from Dr. M. Stedham of Tracor Jitco and was intended to aid Dr. E.E. McConnell in the evaluation of the results of this bioassay. This task was completed.

J. The contractor was requested to prepare fifteen recut slides for each of several tissues from two studies which were previously performed by the contractor. The request was for recuts of two pituitary lesions from the study of the aged ACI rat and for four pituitary and one adrenal lesion from the study of the aged Sprague-Dawley rat. This task has been completed and the slides submitted to Dr. Ward for inclusion in study sets.

K. The EM laboratory of the contractor has completed the processing and evaluation of liver specimens from two 136 week old female Fischer (F344) rats submitted by NCI. The report prepared by the contractor's electron microscopist/pathologist characterized the morphologic characteristics of the basophilic foci which had been diagnosed in the liver specimens by light microscopy.

L. Lastly, a lung neoplasm and adrenal neoplasm from control rats used in a nonbioassay study are being processed and will be evaluated by electron microscopy at the request of Dr. Ward.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This animal pathology support contract provides a great deal of flexibility to the National Toxicology Program contractors and to the Chief of the Chemical Pathology Branch. The personnel and facilities of the contractor are available to the program contractors and to various intramural investigators in the Program. The pathology services provided by the contractor allow for the timely completion of the histopathologic evaluation of sensitive compounds under investigation within the National Toxicology Program. Intramural investigators within the National Toxicology Program may use the facilities and personnel of the contractor to assist them in the conduct of their research programs. The contractor pathologists are available for consultation, and the histology laboratory is available for the processing of animal tissues for histologic examination. Similarly, the histology laboratory is available for the training of intramural and extramural histology technicians.

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. - Herndon, Virginia 22070

NO1-CP-95647

TITLE: Pathology Support for the Carcinogenesis Testing Program (Task II)

CONTRACTOR'S PROJECT DIRECTOR: Dr. J.F. Hardisty and Dr. W.O. Iverson

PROJECT OFFICER (NIEHS): Dr. E.E. McConnell, Chief, Chemical Pathology Branch

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: To assure the quality of pathology arising out of subchronic and chronic bioassays performed under the auspices of the National Toxicology Program. This includes studies conducted under the prime contract (Tracor Jitco) and those conducted under the NTP Basic Ordering Agreement.

METHODS EMPLOYED: The contractor verifies tissue counts and evaluates slide (histological) quality and evaluation of the initial pathologist. The contractor re-examines all tumor diagnoses by the original pathologist, all target tissues and all tissues from 10% of animals chosen at random. When discrepancies are found between the diagnosing and initial pathologists diagnoses, these findings are forwarded to the NTP Pathology Working Group for final evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: During the period October 1, 1981 to September 30, 1982 the chemicals subjected to QA were :

Chemical Name	Chemical Number
1,1,1,2-Tetrachloroethane	C52459
Pentachloroethane	C53894
Allyl Isothiocyanate	C50464
2-Biphenylamine HCL	C50282
Mannitol	C50362
Ziram	C50442
Ascorbic Acid (B6C3F1 Mice)	C54808
BCPE	C50044
Melamine	C50715
Propyl Gallate	C50588

Chemical Name	Chemical Number
Diallylphthalate	C50657
Tetrachloroethylene	C04580
Trichloroethylene	C04546
Telone	C03985
Melamine	C50715
4,4'-Methylenedianiline Dihydrochloride	C54604
Ascorbic Acid (Fischer 344 Rats)	C54808
Benzyl Acetate	C06508
Trichloroethylene (Marshall Rats)	C04546
2,4-Toluene Diisocyanate	C50533
Geranyl Acetate	C54728

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This quality assurance insures that the pathology arising out of studies conducted under the auspices of the NTP are valid and will stand up to peer review.

CLEMENT ASSOCIATES, INC. - Arlington, Virginia 22209

NO1-CP-95646

TITLE: Pathology Support for the Carcinogenesis Testing Program

CONTRACTOR'S PROJECT DIRECTOR: Dr. Dawn Goodman

PROJECT OFFICER (NIEHS): Dr. E.E. McConnell, Chief, Chemical Pathology Branch

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: To assure the quality of pathology arising out of subchronic and chronic bioassays performed under the auspices of the National Toxicology Program. This includes studies conducted under the prime contract (Tracor Jitco) and those conducted under the NTP Basic Ordering Agreement.

METHODS EMPLOYED: The contractor verifies tissue counts and evaluates slide (histological) quality and evaluation of the initial pathologist. The contractor re-examines all tumor diagnoses by the original pathologist, all target tissues and all tissues from 10% of animals chosen at random. When discrepancies are found between the diagnosing and initial pathologists diagnoses, these findings are forwarded to the NTP Pathology Working Group for final evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: During the period October 1, 1981 to September 30, 1982 the contractor has been working on the following tasks:

A. Microslide study set on "Common Lesions in Aging B6C3F1 and BALB/c Mice".

B. Microslide study set on "Chemically Induced Lesions in Rats".

C. Microslide study set on "Induced Lesions in Mice".

D. Evaluation of slides from the Eppley hamster bioassay on furyl furamide.

E. Review of target organs from the Eppley rat bioassay on metronidazole.

F. Review of slides from the Eppley mouse bioassay on succinic acid 2,2-dimethylhydrazine.

G. Evaluation of slides of lungs from the bioassay on male mice administered dimethyl terephthalate.

H. Literature review and evaluation on proliferative lesions of the adrenal gland leading to a workshop on classification of these lesions.

I. Quality assessment of a chronic bioassay done by EPL on 2,6-xylidine in CRL:COBS(CD)SD(BR) rats.

J. Journal article on "Neoplastic and Nonneoplastic Lesions in Aging Osborne-Mendel rats".

K. Journal article on "Chemicals Inducing Splenic Sarcomas in Rats".

L. Journal article on "Chemicals Inducing Nasal Tumors in Rats and Mice".

M. Pathology Workshop.

N. Pathology Working Group

O. Visit to NTP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This quality assurance insures that the pathology arising out of studies conducted under the auspices of the NTP are valid and will stand up to peer review.

PROGRAMS RESOURCES BRANCH

Annual Report of the Program Resources Branch
Toxicology Research and Testing Program

The Program Resources Branch provides essential aspects of toxicology and carcinogenesis studies, namely Animal Care, Analytical Chemistry, and Health and Safety, to the National Toxicology Program. Branch staff procure, analyze and reanalyze chemical compounds for these studies as well as supply disease-free rodents to serve as the test system. The chemical safety group monitors each laboratory and each study within a test facility for those factors which preclude maintenance of the proper research and testing environment. Each resource is provided by substantial in-house effort and supplemented by resource contracts.

The Branch procured or synthesized and completely analyzed fifteen chemical compounds for the general in vivo toxicology studies. Nineteen additional compounds were analyzed for other programs within the National Toxicology Program such as teratology studies, immunotoxicology studies, and continuous breeding experiments. Tissue and body fluid residue analyses were delveloped and performed to enhance data from toxicity experiments of four chemical compounds. Nine-hundred aliquots from approximately 400 compounds were dispensed and shipped to labs under contract to the genetic toxicology program.

The Branch maintains repositories for chemical compounds which are currently under test or which have completed testing in the in vivo bioassay portion of the program and in the genetic toxicology portion of the NTP. Over 1200 individual chemical compounds from these testing programs are stored in these repositories.

In addition to procuring and analyzing chemical compounds for the various subprograms of the NTP, the Program Resources Branch assists the testing facilities in the safe handling of these potentially toxic and carcinogenic substances. Safety aspects, such as flash point determinations, permeation of glove materials, transfer of gases to small bottles, etc., are tested and considered before animal testing commences. Baseline safety evaluations of each facility contracted to the NTP are updated to ensure human health and the integrity of each study are not jeopardized. Each of the over forty laboratories are visited and surveys made to assist in toxicology research and testing.

Over 100,000 disease-free rodents (B6C3F1 mice and F-344 rats) were produced in FY 1982 for use in toxicology testing by the NTP. An innovative room sized isolator was tested, and found acceptable, for production of rodents which are free of infectious disease. This accomplishment brings the production of proper research animals into the reach of several breeding facilities. In the near future, a copious supply of healthy animals can be produced for biological research of all types.

Shipments of mice from two of our contract breeding facilities was halted briefly upon discovery of genetic heterogeneity in the foundation stocks of one of the inbred lines. Genetic monitoring of production animals has become an important aspect of our surveillance program. Genetically pure, disease-free rodents are now produced for bioassay studies.

CHEMICAL REPOSITORIES

The NTP Chemical Repository program provides repository assistance to the Carcinogen Bioassay, Cellular and Genetic Toxicology, Chemical Pathology and the Systemic Toxicology Programs. This includes not only storage but location and acquisition of approximately 1,000 chemicals with a final capacity of 5,000 chemicals, cataloging of Wisweisser Line Notations (WLNS), Chemical Abstract Service (CAS) numbers and numerous other pertinent information on chemical, physical and toxicological properties. This information is input into a custom designed computer program which also generates randomized codes for various aliquots that are to be tested blind. Tracking and monitoring of repository functions are accomplished by this computerized data base management system which allows multi-tier access into a hierarchical system of data retrieval and file security. The repository, upon searching through on-line computer data bases, edits and produces chemical specific handling documents both for day to day safe handling as well as for emergency situations, performs quality assurance (QA) tests for numerous NTP contract laboratories and ships these chemicals to national as well as international laboratories. Finally, upon completion of the biossay studies the repository receives and aliquots an archive sample of the chemical, then arranges for the environmentally safe disposal of surplus test chemicals. The NTP Chemical Repository is thus involved in the entire toxicity testing cycle from beginning to end. Two repositories have been established which operate under similar standard operating procedures; both divide the chemicals into 3 groups, when quantities permit: a testing lot, an archive sample and a public allotment. Present contractural requirements prohibit combination of the Repositories at this time.

FY 1982 ACCOMPLISHMENTS

More than 1,000 aliquots (see Table 1) were sent in FY 1982 to investigators either as coded blind samples or as uncoded shipments for toxicity testing. This is a 50% increase in Repository shipments primarily due to expansion of NTP Cellular and Genetic Toxicology efforts. Redesigned chemical specific Safe Handling Documents and Emergency Procedure Documents have provided clearer and more explicit handling procedures for these potentially toxic test compounds.

Flash point determinations of liquid test chemicals were initiated to meet DOT shipping requirements where this information was unavailable from the literature. A total of 50 compounds have been tested by the closed cup method.

A method of direct probe insertion mass spectrometry was developed to enable impurities separated by high performance thin layer chromatography (HPTLC) to be identified.

An apparatus for determining the permeation of glove materials by NTP test chemicals has been constructed and validation of the method by ASTM standards is completed. The purpose of this work is to provide valuable information to help enable NTP laboratory researchers to conduct studies on test chemicals in a safe

manner. Glove permeability tests on 20 compounds with 4 different glove materials have been studied.

A method for gas transfer from large cylinders to smaller lecture bottles was accomplished and monitored using sulfur hexafluoride as a marker compound. The design of this sampling procedure has allowed effective guidelines to be formulated for the safe handling of gaseous samples.

More effective and explicit packaging and shipping requirements were written to permit transfer of approximately 60 shipments of bulk chemicals to the repository for removal of archive samples and final disposal of surplus bioassay chemicals.

Completion of an addition to the Hazardous Materials Laboratory will now enable storage of approximately 5000, 500-gram samples at 4 different temperature levels (25°C, 5°C, -20°C and -70°C). A safe is also available for storage of narcotics. (Full Drug Enforcement Administration (DEA) license has been approved). (CONTACT PERSON: Dr. D. B. Walters).

FY 1983 PLANS

During FY 1983 the Chemical Repository Program will continue to provide assistance to all branches of NTP.

Increased shipments are anticipated due to the expanded High Priority testing battery of the Cellular and Genetic Toxicology Program.

A more refined computer software and hardware package for tracking and monitoring is being investigated to meet the expanding needs of the NTP.

It is anticipated that approximately 50 compounds will be tested for glove permeability to assist in development of possible structure activity relationships.

Combination of the repositories has been delayed due to contract restraints but plans are proceeding to accomplish this at the end of FY 1984. (CONTACT PERSON: Dr. D. B. Walters).

TABLE 1

NTP CHEMICAL REPOSITORY HOLDINGS AND ACTIVITIES IN FY 1982

	Cellular and Genetic Toxicology Repository	Toxicology and Carcinogenesis Bioassay Repository
1. Control chemicals in inventory	17	--
2. Total number of unique chemicals	856	440
3. Test chemicals shipped for *Salmonella* testing in FY 1982	360	--
4. Test chemicals shipped for *Drosophila* testing in FY 1982	90	--
5. Test chemicals shipped for cytogentics testing in FY 1982	90	--
6. Test chemicals shipped for aneuploidy testing in FY 1982	15	--
7. Test chemicals shipped for Cellular and Genetic Toxicity testing other than 3, 4, 5, 6	350	--
8. Aliquots shipped in FY 1982	905	125
9. Chemicals synthesized	1	--
10. Purity Analyses performed	40	--
11. Quality Assurance samples analyzed	5	5
12. Aliquots transferred to C & GT from T & CB Repository	--	29
13. Chemicals selected for FY 1982 *Salmonella* testing	140	--
14. Chemicals selected for FY 1982 *Drosophila* testing	55	--
15. Chemicals selected for FY 1982 Cytogenetics testing	62	--

	Cellular and Genetic Toxicology Repository	Toxicology and Carcinogenesis Bioassay Repository
16. Number of chemicals analyzed for flash point	50	--
17. Number of chemicals tested in glove permeability study	20	--
18. Number of chemicals analyzed for solubility	330	--

CHEMICAL HEALTH AND SAFETY

The scope of activities in the NTP is necessarily broad and involves research and testing of wide ranges of potentially hazardous materials. The safety program guards against excessive exposure of laboratory personnel and the surrounding environment to the test materials, metabolites, and degradation products. In addition, every effort is made to integrate and coordinate the NTP health and safety requirements and guidelines with the operational work practices used in toxicity testing procedures to minimize any impact on the timeliness, efficiency and effectiveness of performing NTP research in a safe fashion.

Each contract laboratory develops and implements their own health and safety and environmental protection programs. The NTP chemical health and safety program sets standards, establishes guidelines and recommendations and ensures that pertinent local, state, and federal regulations are followed. In addition, the chemical health and safety program has responsibility for monitoring and evaluating the effectiveness of individual laboratories' compliance to established standards, and renders advice in the event of emergency situations. The evaluation of chemical specific health and safety guidelines, safe handling documents and emergency procedures documents for all NTP test chemicals also constitutes a significant part of the program's functions.

The NTP chemical health and safety concerns differ considerably from those of academic or production facilities and require basic understanding and experience in chemistry, industrial hygiene, engineering control, non-routine specialized analytical chemistry, biological monitoring and medical surveillance, human factors and ergonomics, personal protective equipment evaluation and development, sampling strategies and biological testing procedures.

FY 1982 ACCOMPLISHMENTS

Annual health and safety program reviews and site visits were conducted at all carcinogen bioassay contract laboratories. An evaluation as well as a description of action items were submitted to each laboratory with recommendations for corrections.

Two laboratories were monitored for specific test chemicals during industrial hygiene surveys. Five laboratories were monitored for formaldehyde levels involved in necropsy and tissue trimming operations which were observed during baseline health and safety surveys. A study was initiated using fluorescein to compare weighing procedures commonly used in in vitro research utilizing balances placed in hoods versus placement on open laboratory benches. The results were used as a basis for formulation of NTP minimium health and safety requirements for Cellular and Genetic Toxicology laboratories. Gaseous marker chemicals representing chemicals scheduled for Cellular and Genetic Toxicology testing were monitored at the Repository and the test laboratory prior to testing of the more hazardous compounds (e.g. hydrogen cyanide). As a result a revised protocol was suggested.

Waste disposal procedures at all Carcinogen Bioassay laboratories were surveyed and reviewed for consistancy and compliance with RCRA regulations. The high temperature system used for incineration of NTP surplus carcinogen bioassay

chemicals was evaluated for efficacy in addition to evaluation of procedures used for disposal of chemicals not amenable to incineration. An on-site Bioassay contract incinerator was evaluated for possible use for combustion of PBB's and determined to be inadaquate; alternate disposal recommendations were provided. NTP requirements for shipment to the Repository of surplus bioassay chemicals scheduled for disposal were instituted.

Preliminary development studies have been completed for chemical-specific biological monitoring to assist medical surveillance of carcinogenesis bioassay laboratory personnel using 15 test chemicals as markers. Additional work is under consideration pending review of the results and recommendations of the study.

Approximately 40 Cellular and Genetic Toxicology, Carcinogen Bioassay, Pathology and Program Resources contract laboratories have been visited and baseline health and safety surveys were conducted with recommendations provided. Mechanisms are continuing for the health and safety evaluation, on-site inspection and monitoring, where necessary, of NTP laboratories which handle potentially hazardous materials. Plans have been instituted for evaluation and inspection of new laboratories as they join the program as well as periodic reinspection of current contractors. Guidelines and minimum requirements have been approved for all NTP programs except Systemic Toxicology, which is planned for FY-83. These minimum reuirements will be used in support of NIH Guidelines for Laboratory Use of Chemical Carcinogens, OSHA requirements, and other pertinent federal, state and local regulations.

Approximately, 25 chemical specific health and safety guidelines have been prepared, reviewed and evaluated for new test chemicals and have been distributed to the respective chemical managers and pertinent personnel.

A project was initiated to improve the fundamental design of tissue trimming stations in order to provide better capture of formaldehyde, fixatives and trace quantities of potentially hazardous test chemicals. The resultant design incorporated ventilation and ergonomics and can be applied to other work stations such as histopathology and necropsy.

An extensive study of the complex local exhaust ventilation system employed at a bioassay laboratory was undertaken as a result of potentially hazardous conditions. Modifications necessary to improve and rectify existing problems were suggested.

Rapid response to an emergency situation was initiated, in one instance to alleviate potentially hazardous conditions at a Cellular and Genetic Toxicology laboratory.

FY 1983 PROGRAM PLANS

The evaluation, program review, site visit, inspection, monitoring and other programs described as FY 1982 accomplishments will be continued. Health and safety evaluations and on-site inspections of all NTP contract laboratories are planned. Chemical specific health and safety guidelines for new NTP test chemicals will be prepared and reviewed as necessary in addition to evaluation and inspection of new NTP contract laboratories. Efforts to improve and finalize guidelines and minimum requirements for all NTP contract laboratories will be

completed for existing programs. A program for in-house NTP research laboratories to be visited, evaluated and, where necessary, monitored will be instituted with close coordination with NIEHS safety personnel. Plans for the formulation of health and safety guidelines and requirements for in-house research will be considered. In addition, plans call for inspection of all Systemic Toxicology contract laboratories and formulation of health and safety guidelines and minimum standards for these laboratories. Waste management procedures for surplus NTP chemicals will be closely monitored to ensure that all potentially hazardous waste products are disposed of properly and in accordance with RCRA regulations. Studies will continue on program specific human factors and ergonomic evaluations at particular laboratories in seeking ways to improve worker safety coupled with worker effectiveness. A survey of NTP training efforts will be conducted and plans to assist and fulfill training needs will be initiated. (CONTACT PERSON: Dr. D. Walters, NIEHS).

RADIAN CORPORATION - AUSTIN, TEXAS
(NO1-ES-1-5010)

TITLE: National Toxicology Program Chemical Repository

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D., D. R. Boline, Ph.D.

PROJECT OFFICER (NTP): Douglas B. Walters, Ph.D., Program Leader/Chemical Health and Safety, NTP

DATE CONTRACT INITIATED: September 28, 1981

CURRENT ANNUAL LEVEL: $545,702

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for up to 5000 compounds which are to be screened for mutagenicity in the National Toxicology Program. Available physical, chemical and toxicological information is provided on all compounds either from on-line computer data bases or from data collected in the laboratory of the repository. In addition to a testing lot, an archive and a public sample for each test chemical is stored in the repository. Chemical analyses are performed when required.

METHODS EMPLOYED: The repository receives a listing of chemicals which are to be tested either blind or as knowns by laboratories under contract. Upon location and acquisition of these chemicals, the repository searches through on-line computer data bases, edits and produces chemical specific handling documents both for day to day safe handling of the compounds by the testing laboratories as well as for emergency situations. Pertinent information on chemical, physical and toxicological properties is input into a custom designed computer program which also generates randomized codes for the various aliquots that are to be tested blind. Tracking and monitoring of repository functions are accomplished by this computerized data base management system which allows multi-tier access into a hierarchical system of data retrieval and file security. The compounds are doubly contained and shipped according to DOT regulations by safe, appropriate and most expedient possible route to the testing laboratory at a rate of about 75 compounds per month including controls. An estimated 10% of the Salmonella test compounds are analyzed for trace chemical impurities. One compound (N-ethyl-N-nitrosourea) has been synthesized for special NTP needs.

MAJOR FINDINGS AND PROPOSED COURSE: Approximately 900 aliquots have been shipped for FY 1982 testing. Currently, inventory at the repository is over 850 unique chemicals, which are stored in the contractor's Hazardous Materials Laboratory.

Flash point determinations of liquid test chemicals were initiated to meet DOT shipping requirements where this information is unavailable. Approximately 50 compounds per year are be tested by the closed cup method.

An apparatus for determining the permeation of glove materials by NTP test chemicals has been constructed and validation of the method by ASTM standards is

completed. This work provides valuable information to help enable NTP laboratory researchers to conduct studies on test chemicals in a safe manner.

Completion of an addition to the Hazardous Materials Laboratory will now enable storage of approximately 5000, 500-gram samples at 4 different temperature levels (25°C, 5°C, -20°C and -70°C). A safe is also available for storage of narcotics. (Full Drug Enforcement Administration (DEA) license has been approved).

A method for gas transfer from large cylinders to smaller lecture bottles was accomplished and monitored using sulfur hexafluoride as a marker compound. The design of this sampling procedure has allowed effective guidelines to be formulated for the safe handling of gaseous samples.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a comprehensive testing system for mutagenesis (as well as other toxicity testing) requires a repository which can be computerized for effectiveness and efficiency and which is designed around a specially designed containment laboratory for handling hazardous materials. The laboratory also must provide for routine chemical assay as well as sophisticated, complete, chemical trace impurity analysis. These requirements are necessary to support *in vitro* and *in vivo* testing.

PUBLICATIONS

McKinney, J.D., Albro, P.A., Cox, R.H., Hass, J.R., and Walters, D.B.: Problems and Pitfalls in Analytical Studies in Toxicology, in the Pesticide Chemist and Modern Toxicology, Chapter 25, ACS Symposium series, Washington, D.C., 1981.

Boline, D.R., Keith, L.H., Walters, D.B.: Inventory Management and Data Storage for Chemicals Used in Coded Toxicity Testing in *Chemistry Requirements for Toxicity Testing*. Ann Arbor Science, Ann Arbor, Michigan (in press).

Harless, J.M., Walters, D.B.: Design Considerations for a Toxic Chemicals Handling Laboratory in *Health and Safety Recommendations for Toxicity Testing*. Ann Arbor Science, Ann Arbor, Michigan (in press).

Jameson, C.W., Walters, D.B.: *Chemistry Requirements for Toxicity Testing*. Ann Arbor Science, Ann Arbor, Michigan (in press).

Fawkes, J., Albro, P.W., Walters, D., McKinney, J.D.: *Comparative Study of Two Methods for Determining Polybrominated Biphenyl Residues in Animal Tissue*. Analytical Cehmistry (in press).

Title: Rodent Production Contracts

PROJECT OFFICER (NTP): Dr. Charles K. Grieshaber

CO-PROJECT OFFICER: Mr. Clarence Reeder/NCI

DATE CONTRACT INITIATED: September 30, 1979

CHARLES RIVER BREEDING LABORATORIES, PORTAGE — NO1-CP-95641
CONTRACTOR"S PROJECT DIRECTOR: Sumner J. Foster
CURRENT ANNUAL LEVEL: $273,844

CHARLES RIVER BREEDING LABORATORIES, STONERIDGE, NY — NO1-CP-95617
CONTRACTOR'S PROJECT DIRECTOR: Sumner J. Foster
CURRENT ANNUAL LEVEL: $282,413

SIMONSEN LABORATORIES, INCORPORATED, GILROY, CA — NO1-CP-95643
CONTRACTOR'S PROJECT DIRECTOR: Harry Simonsen
CURRENT ANNUAL LEVEL: $120,392

FREDERICK CANCER RESEARCH FACILITY, FREDERICK, MD
PROJECT DIRECTOR: Joseph Mayo
CURRENT ANNUAL LEVEL: $275,000

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to produce disease-free, genetically defined F-344 rats and B6C3F1 hybrid mice for the NTP Bioassay Program.

METHODS EMPLOYED: Genetically defined inbred pedigree stock is obtained from the NIH repository and shipped to each breeding facility. Offspring from matings between these animals are cesarean derived, maintained in isolators, and given defined bacterial flora. These animals serve as inbred foundation stock for each breeding laboratory. Offspring from the foundation stock are used to supply production colonies from which a contract specified number of each species and sex are produced weekly. Rodents are shipped to bioassay contract testing facilities one week after weaning. Pedigreed production breeder stocks are replaced every 30 weeks from the isolator foundation colonies.

MAJOR FINDINGS AND PROPOSED COURSE: The F-344 rat and the B6C3F1 mouse serve as the primary testing systems for the NTP bioassay programs. For FY 1982, 27,000 mice and 28,000 rats were produced for and utilized by the testing program. No chemical starts were delayed more than two weeks due to shortages of test rodents. The animals produced continue to be free of infectious diseases.

Shipment of mice from the Charles River Facilities was halted briefly due to the discovery of genetic heterogeneity in the C3H foundation and production stocks. Genetic monitoring has become an important aspect of the surveillance program. Each generation of inbred stock at all production facilities is monitored for genetic integrity as well as for rodent diseases.

Simonsen laboratories has installed an innovative germ-free room sized isolator for maintaining production of hybrid mice and F-344 rats. This setup will enable production of approximately 200 animals per sex per species per week in a microbiologically defined environment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The NTP has the mandate to assess the toxic and carcinogenic potential of chemical compounds found in the environment. Disease-free, genetically defined F-344 rats and B6C3F1 mice are the primary test system in the NTP Bioassay programs.

RADIAN CORPORATION - AUSTIN, TEXAS
(NO1-CP-95649)

TITLE: Chemical Repository for the National Toxicology Program

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $168,359

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for the chemicals which are studied by the National Toxicology Program. All available physical-chemical and toxicological information is provided on all chemicals.

METHODS EMPLOYED: The contractor receives chemicals which have been or will be tested by the National Toxicology Program. Information is compiled for safety and handling documents as well as complete data sheets on physical-chemical properties, structure, name, toxicology and miscellaneous information. The materials are divided into appropriate aliquots, indexed, cross referenced and inventoried into a computerized system. The chemicals are available to both NTP and non-NTP investigators who may have a need for a sample of a compound which has been tested by the NTP.

MAJOR FINDINGS AND PROPOSED COURSE: The current inventory at the chemical repository is 440 chemicals. All chemicals are stored in the Contractor's Hazardous Materials Laboratory. Catalog file data (chemical properties, etc.) are compliled as compounds are placed in inventory status. Approximately 150 aliquots of chemicals have been shipped in FY 82. Also, approximately 85 aliquots of chemicals were transferred to the NIEHS Chemical Repository for Mutagenicity Screening. In addition, bulk quantities of 96 chemicals were received at the Repository upon completion of testing for the NTP. Repository samples of these surplus chemicals were retained and the excess is delivered to a chemical disposal firm for disposal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of enviromental chemicals. The objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The maintance of a chemical repository to serve as a central archive for the storage and distributation of chemicals studied by the NTP is an essential part in maintaining the quality of the Program.

MIDWEST RESEARCH INSTITUTE - KANSAS CITY, MISSOURI
(NO1-CP-95615)

TITLE: Chemical Servies Support for the National Toxicology Program

CONTRACTOR's PROJECT DIRECTOR: E. J. Woodhouse, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $2,487,238

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to provide chemical procurement, analysis, storage, repackaging, and distribution services in support of the activities of the National Toxicology Program. The contractor serves as an analytical resource for the NTP performing analysis of chemicals for identity, purity assay and stability; formulation of protocols for chemical mixes; analysis of feed samples for toxic components and analysis of dose-feed samples. Special tasks also include isolation and identification of impurities, tissue residue analyses and other associated analytical problems.

METHODS EMPLOYED: The contractor procures and receives chemicals which are to be tested by various Programs in the NTP including both contract and in-house laboratories. Data is generated on the identity and purity of each test material. In addition stability assays are performed to determine the long term stability of both the bulk chemical and chemical-vehicle mixtures. Also, methods are developed for the assay of the test material in the vehicle used in the toxicity test. Other assays such as tissue residue analysis and identification of minor impurities are performed as required.

MAJOR FINDINGS AND PROPOSED COURSE: For FY 82, 15 chemicals were procured or synthesized and analyzed for carcinogenesis bioassay testing. In addition, two new chemicals were procured and analyzed for teratology studies. Analytical services were provided for the NTP's Continuous Breeding Program of the Reproductive Toxicology Section by procuring and analyzing eight new chemicals for study and providing routine analytical chemistry services for the contract labs. Support for other NTP Programs, including immunology and chemical disposition was also accomplished with the procurement and analysis of nine new chemicals. Work was also completed on the tissue and body fluid residue analysis for four chemicals being studied by various members of the intramural staff and contractors for the NTP. Future plans include continued support of the above mentioned activities as well as support of a new NTP initiative for a short term in vivo screening system using the Fischer 344 rat liver as a model. A contract for this initiative is expected to be awarded in early FY 83.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of environmental chemicals. The objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The procurement and analysis of chemicals is one of the essential

steps in the success of bioassays. Without this activity, no substantive animal or in vitro testing could occur. The precise definition of the chemical nature of compounds is one of the cornerstones in an effort to increase the accuracy and reliability of data obtained in toxicological research.

A.D. LITTLE, INC, - CAMBRIDGE, MASSACHUSETTS
(NO1-CP-05673)

TITLE: Health and Safety Services Support for the Carcinogenesis Testing Program

CONTRACTOR'S PROJECT DIRECTOR: R. Scott Stricoff

PROJECT OFFICER (NTP): Douglas B. Walters, Ph.D., Program Leader/Chemical Health and Safety, NTP

DATE CONTRACT INITIATED: September 30, 1980

CURRENT ANNUAL LEVEL: $305,453

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to assist the National Toxicology Program in the evaluation of health and safety practices of the NTP and its contract laboratories. Assistance provided includes: basic industrial hygiene as well as specialized experience in analytical chemistry, engineering control, personal protective equipment evaluation, human factors evaluation and statistical design of sampling strategies.

METHODS EMPLOYED: The Contractor furnishes services, qualified personnel, material, equipment and facilities as needed to evaluate, survey and assist the NTP in the following areas:

Design, implementation, and qualitative and quantitative evaluation of industrial hygiene and pollution monitoring programs at designated facilities to examine the spread of NTP test materials.

Development and evaluation of alternate work practices or engineering controls in alleviating potentially hazardous situations encountered in NTP facilities.

Inspection of laboratory facilities for qualitative evaluation of the health and safety program.

Development of Baseline Health and Safety Surveys of NTP facilities.

Preparation of chemical specific Health and Safety documents for use by NTP contract personnel.

Response to special situations requiring rapid action because of potentially hazardous conditions.

Evaluation of Incinerator Design for proper disposal of various NTP toxicity testing chemicals.

MAJOR FINDINGS AND PROPOSED COURSE: Coordination and planning meetings have occured and include the health and saftey evaluation of: NTP contract laboratories in the Cellular and Genetic Toxicology program and all other NTP testing efforts; procedures used for disposal by incineration of surplus NTP

chemicals; medical monitoring programs applicable to NTP Carcinogenesis Bioassay laboratories; the health and safety programs; procedures and facilities of current carcinogenesis bioassay laboratories via participation and assistance in the routine monitoring and inspection program: practices and equipment used for personal protection.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of environmental chemicals. The objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The maintenance of an effective Health and Safety Program is an essential part in maintaining the quality of the Program.

PUBLICATIONS

Walters, D.B.: Chemical Health and Safety for Toxicity Testing: An Overview in Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Walters, D.B., Stricoff, R.S., Harless, J.M.: Chemical Containment: Design Criteria for Toxicity Testing Facilities in Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Stricoff, R.S., Hoyle, E.R., Walters, D.B.: Health and Safety in the Design of Toxicity Testing Laboratories in Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Prokopetz, A.T., Prescott, E.M., Walters, D.B.: Preparation of Chemical Health and Safety Documents in Health and Safty Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Beres, J.J., Walters, D.B.: Industrial Hygiene Monitoring of Chemical Contaminants at Bioassay Laboratories in Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Phelan, E.J., Walters, D.B.: Human Factor Considerations in the Handling of Toxic Chemicals in Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Walters, D.B., Jameson, C.W.: Health and Safety Recommendations for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

PROGRAMS OPERATIONS BRANCH

PROGRAM OPERATIONS BRANCH
Summary Statement

The Program Operations Branch has the primary responsibility within the National Toxicology Program (NTP) to oversee that the relevant scientific decisions are converted into effective extramural laboratory studies. This includes the translation into agreements with testing facilities through the Collaborative Services Group, monitoring of the experiments by the Test Management Group, verification of the information developed by the Quality Assurance - Good Laboratory Practices Group, and an integration of the NTP facets needed for an efficacious operation by the Planning and Coordination Group. The Branch is also charged with the responsibility of overseeing the phase-out of the Prime Contractor.

In addition to its prime functions, members of the Branch participate in other NTP functions as chemical managers, special toxicology consultants, etc.

Collaborative Services

Nineteen laboratories successfully competed and were awarded Master Agreements (MA's) for the possible conduct of bioassays on chemicals in rodents for toxicologic/carcinogenic potential. The pool of qualified laboratories now stands at 19 as listed in the attached Table 1 and 1A. It is anticipated that the Master Agreement will be readvertised during the current fiscal year. The Master Agreement has been completely revised and is constantly undergoing modifications to reflect current state-of-the-art technology.

Nineteen chemicals arranged in 11 packages have been competed among the laboratories in the pool judged qualified to conduct bioassays under the Master Agreement referenced above. Task Order awards to six laboratories were completed during FY 81 for a total of $3,980,116 for the conduct of bioassays on the chemicals listed in Table 2. It is anticipated that task orders for tests on approximately 11 more chemicals will be placed during this fiscal year.

New contracts have also been negotiated for cell transformation assays and multiple endpoint mutation studies as well as modifications of the Salmonella test for chemicals that may be metabolized to mutagens under reductive conditions as shown in Table 3. The total value of these awards was $6,855,417. The studies in these several project plans are aimed primarily at the development and validation of short term in vitro methods. A new Master Agreement for Technical Information Services is under negotiation with awards anticipated during the third quarter of Fiscal Year 1982.

Collaborative Services has also participated in: (1) the phase-over of Tracor Jitco subcontracts, (2) implementing, tracking and monitoring of modifications to the Master Agreement and Task Order awards, and (3) debriefing of unsuccessful offerors.

TABLE 1. Laboratories qualified for basic ordering agreements to conduct carcinogenesis bioassays and other toxicologic and related studies. The special study capabilities of the laboratories are shown in Table 1A.

LABORATORY	ROUTES OF ADMINISTRATION					
	Dosed Feed	Dosed Water	Gavage	Skin Paint	Inhalation	Other
Battelle Columbus	x	x	x	x		Injection
Battelle Pacific-Northwest	x	x	x	x	x	
Bioassay Systems	x	x	x	x		
EG&G Mason Research Inst.	x	x	x	x		
Food & Drug Research Labs	x	x	x	x	x	
Gulf South Research Inst.	x	x	x	x		
Hazleton Laboratories America	x	x	x	x	x	
Illinois Inst. of Technology Research Inst.	x	x	x	x		Intratracheal
International Research and Development Corp. (IRDC)	x	x	x	x	x	
Litton Bionetics	x	x	x	x	x	
Microbiological Associates	x	x	x	x	Nose Only (Mice)	
Midwest Research Inst.	x	x	x	x	x	
Papanicolaou Cancer Res. Inst.	x	x	x	x		
Raltech Scientific Services					x[1]	
Southern Research Inst.	x	x	x	x		Injection
Springborn Inst. for Research	x	x	x	x		
SRI International	x	x	x	x		
Toxicity Research			x			
ToxiGenics					x	
Totals:	16	16	17	16	9	

[1] Acute and 14-Day Repeated Dose Only
[2] Hematology Only

TABLE 1A

LABORATORY	SPECIFIC TOXICOLOGIC PARAMETERS				
	HUCC	Chemical Disposition	Immuno-Toxicology	Neuro-behavioral Toxicology	Endo-crinology
Battelle Columbus	x	x	x	x	x
Battelle Pacific-Northwest	x	x	x	x	x
Bioassay Systems	x	x			
EG&G Mason Research Inst.	x	x	x	x	x
Food & Drug Research Labs	x				
Gulf South Research Inst.	x				
Hazleton Laboratories America	x	x	x	x	x
Illinois Inst. of Technology Research Inst.	x		x	x	x
International Research and Development Corp. (IRDC)	x	x		x	x
Litton Bionetics	x	x	x		x
Microbiological Associates		x	x		x
Midwest Research Inst.	x	x			x
Papanicolaou Cancer Res. Inst.	x[2]				
Raltech Scientific Services	x	x	x	x	x
Southern Research Inst.	x	x			x
Springborn Inst. for Research	x				
SRI International	x	x	x	x	
Toxicity Research	x				
ToxiGenics	x	x			
Totals:	18	13	9	8	10

[1] Acute and 14-Day Repeated Dose Only
[2] Hematology Only

TABLE 2: Chemicals placed on test during FY 81 through Task Order awards under the Master Agreement

Battelle Columbus

1. o-Benzyl-p-chlorophenol (120-32-1)
2. Tricresyl phosphate (1330-78-5)

Gulf South

1. Manganese sulfate (7785-87-7)
2. Tetrahydrofuran (109-99-9)

Hazleton Laboratories America

1. C.I. Acid Red 114 (6459-94-5)
2. 3,3'-Dimethylbenzidine (119-93-7)
3. C.I. Direct Blue 15 (2429-74-5)
4. 3,3'-Dimethoxybenzidine (119-90-4)
5. 1,2,3-Trichloropropane (96-18-4)
6. Acetonitrile (75-05-8)

Hazleton-Raltech

1. p-Nitroaniline (100-01-6)
2. o-Nitroanisole (91-23-6)
3. Methylphenidate (113-45-1)
4. Riddelline (23246-96-0)

International Research and Development Corporation, (IRDC)

1. C.I. Direct Blue 218 (10401-50-0)
2. Triamterene (396-01-0)
3. Propantheline Bromide (50-34-0)

Litton Bionetics

1. Promethazine (60-87-7)
2. Methdilazine (1982-37-2)

TABLE 3: Contract awards aimed at the development and validation of short term *in vitro* methods.

Mammalian Cell Transformation

Task I	Microbiological Associates A.D. Little
Task II	Microbiological Associates Northrup
Task III	Litton Bionetics Northrup Biotech

Development and Validation of a Multiple Endpoint Mutation System in Cultured Mammalian Cells.

Allied Corporation
Bioassay Systems

Modification of the Salmonella Test for Chemicals That May Be Metabolized to Mutagens Under Reductive Conditions

Michigan Cancer

Good Laboratory Practices Compliance

Toxicological and carcinogenesis animal studies for the Bioassay Program initiated under subcontracts through our Prime Contractor, Tracor Jitco, Inc., and through contracts directly with NTP are being done under the Food and Drug Administration's Good Laboratory Practices (GLP) Regulations for Non-clinical Laboratory Studies (Federal Register, December 22, 1978, Part II). Quality Assurance Unit of Tracor Jitco monitors the activities of the Bioassay Subcontracts. A NTP Quality Assurance Leader works with Tracor Jitco and has monitored the GLP practices of the NTP Bioassay Contractor Laboratories. All existing bioassay contractors established under the master agreement for animal toxicology carcinogenicity have been modified to include GLP compliance. Site visits are being made to inspect and provide guidance to the laboratory as to how NTP intends to comply with GLP requirements and will include monitoring of both in-life and data audits.

The analytical chemistry contracts servicing both the bioassay subcontracts through the Prime Contractor, Tracor Jitco, Inc., and through bioassay contracts directly with NTP have been brought under GLP compliance. The chemical repository for the in vitro laboratories has been visited and the requirements for GLP compliance have been evaluated.

The requirements for bringing the bioassay repository and archives under GLP compliance has been evaluated and will be in place by September 30, 1982.

In collaboration with NCTR and NIOSH, all teratological and behavioral toxicology studies carried out under joint funding with NIEHS have been visited during FY 1982 and will be required to comply with FDA GLP's by September 30, 1982.

Those contracts for In Vitro Genotoxicology for validation of testing procedures and for actual testing of chemicals are being reviewed with the intent of developing guidelines for bringing these under GLP compliance in FY 1983.

A study is under way to develop a plan for the audit of data computer collected on the Toxicology Data Management System (TDMS). This has been planned to be in place as this system is adapted by the bioassay laboratories for the chronic studies initiated the latter part of FY 1982 and during FY 1983.

Planning and Coordination

During FY 1982, the Planning and Coordination Group (PCG) has developed and implemented several new tracking procedures to follow the progress of each chemical from nomination through testing to publication. Historical data has been collected for most chemicals currently on test within the NTP. The data base has also been analyzed for special problem areas and updated for accuracy, consistency, and completeness.

PCG has developed a variety of programs during FY 1982 that provide individual NTP staff members with critical information specific to their needs. Individualized groups of chemical information sheets are now provided to project officers and chemical managers on a regular basis. Several specialty programs

have also been developed and are in routine usage; these include an extensive program for projecting chemicals starting and completing various phases of testing during any given quarter, as well as several other types of programs projecting individual workloads during various phases of testing.

PCG continues to provide monthly schedules of all NTP activities as well as collect both historical and current information, refine tracking methodology, monitor test schedule compliance, and develop new programs to optimize effectiveness and coordination among NTP staff.

Test Management

The Test Management Group of the Program Operations Branch is responsible for monitoring toxicological and carcinogenesis studies through direct contracts. The staff (as Laboratory Monitors) also helps in the monitoring of the subcontract laboratories that have contracts through Tracor Jitco as well as monitoring the performance of the Tracor Jitco staff. As Project Officers, the major emphasis in laboratory monitoring is to assure that (1) the contract laboratory has and maintains an acceptable level of facilities, equipment, and scientific, administrative and technical staff; (2) performs satisfactory toxicology and carcinogenesis testing of chemicals in a timely manner; and (3) assures the safety of the personnel assigned to test programs. This is accomplished through scheduled site visits, extensive contacts with the laboratories, reports review, and maintaining communications with other scientists within NTP (Discipline Leaders, Specialty Leaders, Senior Chemical Managers and Chemical Managers) and key contract personnel. The Senior Laboratory Monitor is responsible for these activities through supervision of the project officers and organizational planning and oversight of the monitoring function.

EG&G MASON RESEARCH INSTITUTE:

Principal Investigator: Dr. Herman S. Lilja
NTP Project Officer: Dr. Dexter S. Goldman

Compound	Route	NTP#	Test Stage[d]
Pentaerythritol Tetranitrate [a.1,b]	Feed	10150-E	Prechronic
H.C. Yellow 4 [a.1,b]	Feed	10123-S	Prechronic
Chlorpromazine HCL [a.2,b]	Feed	10101-T	Prechronic
4-Hydroxyacetanilide [a.2,b]	Feed	10127-H	Prechronic
Turmeric, Oleoresin [a.2,c]	Feed	10105-J	Prechronic
1-Amino-2,4-Dibromoanthraquinone [a.3,b]	Feed	10084-N	Prechronic
2,4-Diaminophenol HCl [a.3]	Gavage	10106-N	Prechronic
Probenecid [a.3]	Gavage	10160-L	Prechronic
Quercetin [a.3]	Feed	10161-R	Assigned
Hexachloroethane [a.4,b]	Gavage	10126-E	Chronic
Nitrobenzene [a.4,b]	Gavage/Dermal	10141-A	Prechronic
Titanium Ferrocene [a.4,b]	Gavage	10174-K	Prechronic

Contracts	Date Initiated	Funding Level
a. 1 NO1-CP-95619-01	June 30, 1980	$342,768
2 NO1-CP-95619-02	August 31, 1980	271,353
3 NO1-CP-95619-03	July 31, 1980	312,878
4 NO1-CP-95619-04	August 30, 1980	332,120

b. special studies

c. substituted for curcumin (NCI No. C60015)

d. As of May 1, 1982

BIOASSAY SYSTEMS CORPORATION:

Principal Investigator: Dr. Indu Muni

NTP Project Officer: Dr. Dexter S. Goldman

Compound	Route	NTP#	Test Stage*
Hydroquinone	Dermal	10022-H	Prechronic

* As of May 1, 1982

Contract No.: N01-ES-28022
Date Initiated: May 1, 1982
Current Annual
Funding Level: $263,260

BATTELLE-COLUMBUS

Principal Investigator: Dr. Arthur Peters

NTP Project Officer: Dr. Rajendra Chhabra

Compound	Route	NTP#	Stage
p-Chloroaniline [a.1, b]	gavage	C02039B	chronic
Dimethoxane [a.1, b]	gavage	C56213	prechronic
Ocratoxin A [a.1]	gavage	C56586	prechronic
Ethylenediamine [a.2, b]	gavage	C60402	suspended
Methylolacrylamide [a.2, b]	gavage	C60333	chronic
1-Vinyl-3-cyclohexene dioxide [a.2, b]	gavage	C60139A	prechronic

	Contracts	Date Initiated	Current Annual Funding Level
a.	1 NO1-CP-95653-01	August 31, 1980	$209,296
	2 NO1-CP-95653-02	September 30, 1980	346,263

b. special studies

GULF SOUTH RESEARCH INSTITUTE

Principal Investigator: Mr. Ralph J. Wheeler

NTP Project Officer: Dr. Joseph E. Tomaszewski

Compound	Route	NTP#	Stage
Manganese Sulfate [a.1, b]	dose feed	C61143	repeated dose
Tetrahydrofuran [a.2, b]	gavage	C60560	repeated dose

Contracts	Date Initiated	Current Annual Funding Level
a. 1. NO1-CP-05698-01	September 30, 1981	$74,920
2. NO1-CP-05698-02	September 30, 1981	$63,053

b. Special Studies

HAZLETON LABORATORIES AMERICA, INC.

Principal Investigators: Dr. William Coate [b]
Dr. Borge Ulland [a, b]

Project Officer: Dr. James McCoy

Compound	Route	NTP#	Stage of Test
Acetonitrile [a]	Inhalation	10242-J	90-day subchronic
Trichloropropane [b]	Gavage	10914-F	90-day subchronic
C.I. Acid Red 114 [c]	Water	10286-G	14-day repeated dose
3,3'-Dimethylbenzidine [c]	Water	11045-W	14-day repeated dose
C.I. Direct Blue 15 [c]	Water	10701-A	14-day repeated dose
3,3'-Dimethoxybenzidine [c]	Water	10895-S	14-day repeated dose

	Contracts	Date Initiated	Current Annual Funding Level
a.	NO1-CP-95655-01	8/31/81	$183,337
b.	NO1-CP-95655-02	8/31/81	$178,293
c.	NO1-CP-95655-03	9/30/81	$2,623,258

HAZLETON RALTECH, INC.

Principal Investigator: Dr. Karen MacKenzie
NTP Project Officer: Dr. Carrie E. Whitmire

Compound	Route	NTP#	Stage
o-Nitroanisole [a.2, b]	feed	10211G	prechronic
p-Nitroaniline [a.2, b]	gavage	10217F	prechronic
Riddelliine [a.1, b, c]	gavage	10237W	prechronic
Methylphenidate [a.1, b]	feed	10266R	prechronic

Contracts	Date Initiated	Current Annual Funding Level
a. 1 NO1-CP-05696-01	September 30, 1981	$556,868 (2 yr. fully funded)
2 NO1-CP-05696-02	September 30, 1981	$145,984

b. Special Studies

c. Substituted for Seneciphylline

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Principal Investigator: Dr. Clifford Jessup
NTP Project Officer: Dr. Marcelina B. Powers

Compound	Route	NTP#	Stage
Toluene [a.1, b]	(gavage (inhalation	10009-V	prechronic only (completed) chronic
Isoproterenol, HCl [a.1]	inhalation	10130-N	prechronic
Dimethyloldihydroxyethylene urea [a.1,b]	(inhalation (gavage	10205-P	prechronic prechronic
Coumarin [a.2, b]	gavage	10104-S	prechronic only (completed)
6-Methylcoumarin [a.2, b]	gavage	10136-L	"
3,4-Dihydrocoumarin [a.2, b]	gavage	10113-J	"
Azodicarbonamide [a.3, b]	(inhalation (gavage	10086-W	cancelled after acute chronic
Isobutyl Nitrite [a.3, b]	inhalation	10869-J	prechronic
Carvone [a.4, b]	gavage	10093-S	chronic
Resorcinol [a.4, b]	gavage	10009-V	chronic
Diethylphthalate [a.4, b]	feed	10112-F	cancelled after prechronic
Mercuric chloride [a.5, b]	gavage	10133-A	chronic
Palladium chloride [a.5, b]	gavage	10148-D	prechronic
Monochloroacetic acid [a.5, b]	gavage	10138-V	chronic
Chloramphenicol [a.6, b]	feed	10096-E	prechronic
4,4'-Diamino-2,2'-stilbenedisulfonic acid [a.6]	feed	10107-S	chronic
Cadinene [a.6]	feed	10091-J	cancelled after prechronic

	Contracts	Date Initiated	Current Annual Funding Level
a.	1 NO1-CP-05700-01	August 31, 1980	$646,850
	2 NO1-CP-05700-02	August 31, 1980	$5,399
	3 NO1-CP-05700-03	August 31, 1980	$457,251
	4 NO1-CP-05700-04	October 6, 1980	$749,500
	5 NO1-CP-05700-05	August 31, 1980	$336,121
	6 NO1-CP-05700-06	August 31, 1980	$297,185

b. special studies

LITTON BIONETICS, INC.

Principal Investigator: Dr. Allan G. Manus

NTP Project Officer: Dr. Sandra C. Brown

Compound	Route	NTP#	Stage
Promethazine	gavage	C60673	prechronic
Methdilazine	gavage	C60720A	prechronic

Contract	Date Initiated	Current Annual Funding Level
N01-CP-95652-01	September 30, 1981	$392,962

MICROBIOLOGICAL ASSOCIATES

Principal Investigator: Dr. Marshall Dinowitz

NTP Project Officer: Dr. John A. Quest

Compound	Route	NTP#	Stage
dl-Amphetamine sulfate [a]	feed	C55710	chronic
Sodium Azide [a]	feed	C06462	chronic
Tris (2-chloroethyl) phosphate [a]	gavage	C54751	chronic

Contracts	Date Initiated	Current Annual Funding Level
a. NO1-CP-95650-01	September 29, 1980	$331,101

SOUTHERN RESEARCH INSTITUTE

Principal Investigator: Dr. David Prejean
NTP Project Officer: Dr. William Eastin

Compound	Route	NTP#	Stage
Hexachlorocyclopentadiene [a.1]	gavage	10125-A	cancelled
Benzaldehyde [a.1]	gavage	10087-A	chronic
Gamma-butyrolactone [a.1]	gavage	10090-F	chronic
Furan [a.1, b]	gavage	10119-M	chronic
Furfuryl alcohol [a.1, b]	gavage	10121-J	cancelled
Furfural [a.1, b]	gavage	10120-F	chronic
Ethylene glycol [a.2, b]	feed	C00920	chronic
Pigment Red 3 [a.2, b]	feed	C54922	prechronic
Red 23 [a.2, b]	feed	C60377	prechronic
Polysorbate 80 [a.2, b]	feed	C60286	chronic

Contracts	Date Initiated	Current Annual Funding Level
a. 1 NO-1-CP-95651-01	June 30, 1980	$804,203
2 NO-1-CP-95651-02	September 30, 1980	473,907

b. special studies

01 - for Furan, Furfural and Furfuryl alcohol - metabolism

02 - Hematology, urinalysis and clinical chemistry for Ethylene glycol, CI Pigment Red 3 and CI Pigment Red 23.

TRACOR JITCO, INC. - Rockville, Maryland 20852
(NO1-CP-43350)

TITLE: Bioassay Prime Contract

CONTRACTOR'S PROJECT DIRECTOR: John Keller, Ph.D.

PROJECT OFFICER (NIEHS/NTP): J. Fielding Douglas, Ph.D.
Bioassay Program, NIEHS/NTP

DATE CONTRACT INITIATED: March 1, 1974

CURRENT ANNUAL LEVEL:

PROJECT DESCRIPTION

OBJECTIVES: The purpose of the Bioassay Prime Contract is to provide scientific and management support to the bioassay program in the conduct of carcinogenesis bioassay testing of environmental chemicals. This support entails the following: (1) maintain responsibility for the accurate and timely performance of bioassay subcontracts under the prime; (2) continue to coordinate and monitor the research conducted by the subcontractors; (3) propose and if approved by NIEHS/NTP, carry out scientific improvements and cost-saving management methods for the program; (4) purchase chemicals and obtain chemical analysis information on the chemicals to be tested; (5) provide for data submission to the Carcinogenesis Bioassay Data System (CBDS) and assist in the preparation of final reports on the chemicals tested; (6) continue to evaluate, monitor, quality assess and improve pathology services of the program. Hold workshops to improve diagnoses and overall program effort; (7) maintain best effort in performing other tasks as assigned by NIEHS/NTP.

METHODS EMPLOYED AND MAJOR FINDINGS: Tracor Jitco supports bioassay and toxicological experiments on 135 chemicals in various phases of study. Table 1 lists the chemicals and their status as of 4/26/82. These studies are conducted under subcontracts to 17 facilities and specialized individuals. Table 2 provides the latter information. During the FY 1982, 12 bioassays were completed and technical reports prepared describing the experiments, results and evaluation of the data. The studies were reviewed in public hearings by a peer review subcommittee of the Board of Scientific Counselors. The reports reviewed (all were accepted) were on the following chemicals:

Report	Peer Review Subcommittee Meeting Date
Zearalenone	December 16, 1982
Mannitol	"
Propyl Gallate	"
1,1,1,2-Tetrachloroethane	"
Ziram	"
Bis(2-chloro-1-methylethyl) Ether	"

Diallylphthalate	June 16, 1982
Trichloroethylene	"
Melamine	"
Ascorbic Acid	"
4,4-Methylenedianiline Dihydrochloride	"
Benzyl Acetate	"
To be determined	September 1982

PROPOSED COURSE: It is intended to continue ongoing bioassays of chemicals to completion of the Tracor Jitco Contract, May 31, 1983. The NIEHS/NTP is gradually assuming responsibility for the various laboratories at the approximate rate of one per month. As of June 1, 1982, they have taken direct control of Bioassay Systems Corporation and Southern Research Institute. All results obtained will be reported in the NTP Carcinogenesis Technical Report Series.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the primary factors basic to the understanding and prevention of cancer is the identification of carcinogenic chemicals in the environment of man. If "environment" is defined to include not only the atmosphere, but also industry, transportation, clothing, housing, occupation, food, drugs, cosmetics, and any other entity with which man comes into contact, the number of suspect chemicals is very large. Thus, the number of chemicals which must be tested is large. Because of the nature of the bioassay, numerous animals must be utilized in testing each chemical over long-time periods. Extensive elements of data are produced which must be recorded accurately and interpreted expertly. This convergence of large-dimension factors results in high costs. To obtain the greatest value in knowledge with minimal expenditures of funds, it is essential that the Program be managed carefully to ensure good science, expert use of manpower and funds, and high quality. A principal goal of the Prime Contractor, therefore, is to achieve the objectives of the NIEHS/NTP Bioassay Program with high scientific credibility at reasonable costs.

TABLE 1 - BIOASSAY CHEMICALS IN THE PRIME CONTRACT

A. In Chronic Study

p-Dichlorobenzene
Rotenone
THPS
THPC
N-Phenyl-B-naphthylamine
Xylenes
2,4-Dichlorophenol
Chlorpheniramine maleate
Orthophenylphenol
Sodium fluoride
Pentachlorophenol (technical)

Methyl Methacrylate
Tetrachloroethylene (inhalation)
Ethylene Oxide
Ethyl Chloride
Methylene Chloride (inhalation)
1,3-Butadiene
1,2-Epoxybutane
Ethyl Bromide

Diethanolamine
Sodium xylene sulfonate
Coconut Oil, DEA
Lauric acid, DEA
Oleic acid, DEA
Glutaraldehyde
p-Nitrophenol
2-Butanone Peroxide
1,2-Epoxyhexadecane
Chloramine

Decabromodiphenyl oxide
Chlorendic acid
Dimethyl Morpholinophosphoramidate
Dimethyl Methyl Phosphonate
3-chloro-2-methylpropene
Dimethylvinyl chloride
Diesel fuel marine
Navy fuel JP 5
4-Vinylcyclohexene

C. I. Basic Red 9
Iodinated Glycerol
Bromoform
Phenylbutazone
Bromodichloromethane

Chlorinated trisodium phosphate
Pentachloronitrobenzene
Boric Acid

Benzyl Alcohol
d-Limonene
Succinic Anhydride
Methyl Carbamate
alpha-Methylbenzyl Alcohol

Vinyl Toluene
Tetranitromethane

2,3-Dibromo-1-propanol
Glycidol

2-Amino-4-nitrophenol
Phenylephrine HCl
Tetracycline hydrochloride
Hexylresorcinol
Nalidixic Acid
Nitrofurazone
Mercaptobenzothiazole
Methyldopa
Ephedrine sulfate
2-Amino-5-nitrophenol
Erythromycin stearate
Oxytetracycline

Nitrofurantoin
Acid Orange 3
Chlorowax 40
Chlorowax 500C
Rhodamine 6G
Roxarsone
Dichlorvos

Ampicillin Trihydrate
Penicillin V K
Benzofuran
N,N-Dimethylaniline

Diphenhydramine hydrochloride
8-Methoxypsoralen
Furosemide
Hydrochlorothiazide

TABLE 1 - BIOASSAY CHEMICALS IN THE PRIME CONTRACT (continued)

B. In Prechronic Study

Chlorobenzalmalononitrile and Hexamethyldisilizane (CS2)
Allyl Glycidyl Ether
alpha-Chloroacetophenone
Epinephrine
Hydroquinone
Catechol
p-Quinone
2,2-bis(Bromomethyl)-1,3-propanediol
Pentachloroanisole
Pentachlorophenol (Dowicide EC-7)

C. In the Report Preparation Stage

o-Dichlorobenzene
Chlorobenzene
Malonaldehyde
Fluorescein, sodium
Benzene
Methylene Chloride (gavage)
Tetrachloroethylene (gavage, rat strain study)
1,2-Dichloropropane
Propylene
Propylene Oxide
Sodium (2-ethylhexyl) alcohol sulfate
Maleic hydrazide, diethanolamine salt
Witch Hazel
Sodium dodecylsulfate
Castor Oil
Ethylene Glycol Monoethyl Ether
Pyridine
Trichlorfon
Gilsonite
t-Butyl alcohol
DMBA/TPA
Dodecyl alcohol, ethoxylated
Ethylene Chlorohydrin
Diallylphthalate
Dimethyl hydrogen phosphite
Tris(2-ethylhexyl)phosphate
Monuron
Chlorodibromomethane
Diglycidylresorcinol ether
n-Butyl chloride
8-Hydroxyquinoline
Isophorone
Ethyl acrylate
Trichloroethylene (rat strain study)
1,1,1-Trichloroethane (methyl chloroform)
HC Blue 1
HC Blue 2
HC Red 3
Disperse Blue 1
Geranyl acetate
Allyl isothiocyanate
Toluene diisocyanate
Allyl isovalerate

TABLE 2 - SUBCONTRACTORS TO THE PRIME CONTRACT DURING FY 1982

Battelle-Columbus Laboratories
Battelle-Pacific Northwest Laboratories
Bioassay Systems, Inc.
Bio-Serv, Inc.
EG&G Mason Research Institute
Gulf South Research Institute
Hazleton Laboratories America, Inc.
Litton Bionetics, Inc.
Microbiological Associates
Midwest Research Institute (chemistry)
Midwest Research Institute (inhalation)
NuChem Co., Inc.
Papanicolaou Cancer Research Institute
Physiological Research Laboratories
Southern Research Institute
Springborn Institute for Bioresearch, Inc.
SRI International

SYSTEMIC TOXICOLOGY BRANCH

SYSTEMIC TOXICOLOGY BRANCH
Summary Statement

Comprehensive toxicologic characterization of chemicals in laboratory animals remains as the foundation for predicting with assurance the eventual adverse effects from particular chemicals to humans. These toxicology information dossiers come from the integrated programs within the Systemic Toxicology Branch, and in combination with the experimental data generated in other Branches (Cellular and Genetic Toxicology, Chemical Pathology, Carcinogenesis and Toxicology Evaluation) allow an evaluation to be made on the gamut of toxic effects in animals and form the basis for extrapolating potential hazards to humans.

The Systemic Toxicology Branch comprises five sections: Biochemical Toxicology, Chemical Disposition, Fertility and Reproduction, Immunologic Toxicology, and Inhalation Toxicology. Each is summarized below; for more details and specific accomplishments consult the individual discussions on the following pages.

Biochemical Toxicology -- Studies structure-activity relationships of chemicals to ascertain the mechanism(s) of action(s) at the molecular and biochemical level. Major projects encompass identification and characterization of chemically-induced alterations in cytochromé P-450s. These enzymes catalyze foreign chemical metabolism. The structure-activity effects on the cytochrome P-450s are being defined using the environmental chemicals PCBs and Dioxins (2,3,7,8-TCDD).

Chemical Disposition -- Investigates the absorption, distribution, metabolism, and excretion of a range of chemicals to provide information which will be useful to the design and interpretation of studies of chemical toxicity and carcinogenicity. Studies of chemical disposition are chosen and designed to provide data which will permit a better understanding of the structure-activity relations which determine the rates of chemical absorption, distribution, metabolism, and excretion. Thus these are designed to contribute to our basic understanding of chemical toxicity and will identify those factors which mediate chemical toxicity and will allow a more accurate extrapolation of laboratory data to humans.

Major projects include the metabolism and disposition of benzidine and congener dyes (3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine); determining the biologic impact on disposition patterns from species, sex, age, dose, and route of exposure; and profiling most chemicals entered into the carcinogenesis testing mode.

Fertility and Reproduction -- Provides three major types of support: maintenance of an in-house research program, consultation on the design of special studies, and participation with NIOSH and NCTR personnel in the organization, coordination, and long range planning of the NTP Reproductive and Developmental Toxicology Program.

The in-house research activities include the evaluation of the reproductive toxicity of environmental and industrial chemicals, and provide relevant data on the toxic potential and mechanism of chemicals, enhance the development of new

and more appropriate testing systems, and assure advanced technology in test development and validation.

Two recent major additions center on i) evaluating a comprehensive testing system to assess fertility by continuous breeding and ii) identifying chemicals, using the 90-day exposure bioassay studies, that influence sperm number and morphology in male rodents and vaginal cytology and reproductive cycle in female rodents.

Immunotoxicology -- Selects, refines, and validates a panel of immunology and host resistance procedures to define immunotoxicity and to correlate changes in immune function with alterations in host resistance. The comprehensive assay panel evaluates: (1) host resistance to bacterial, parasitic, and tumor cell challenge; (2) cell-mediated immune functions; (3) humoral mediated immune functions; (4) macrophage functions; (5) natural killer cell activity; and (6) myelotoxicity.

Selected chemicals, most being known or suspected human carcinogens, are tested using these assays to determine (1) the carcinogenic-immunotoxic relation; (2) structure-activity correlations; and (3) mechanism(s) of immunotoxicity.

Inhalation Toxicology -- Conducts studies on compounds to which toxicologically significant exposure occurs primarily by inhalation and works toward the technologic advancement of gas-vapor inhalation methodologies/facilities. The research program divides into three areas: (1) cardiac toxicology using isolated, perfused hearts and other cardiac tissue, (2) carcinogenic potential of simultaneous exposure to nitrogen dioxide and nitrosatable amines, and (3) inhalation toxicology of environmental chemicals.

Studies are underway to examine the effects from co-exposure of morpholine by gavage or drinking water and nitrogen dioxide by inhalation; tissues are analyzed for probable nitroso compounds (N-nitrosomorpholine) and N-nitromorpholine. Carbon disulfide is being investigated to examine the potential acceleration of atherosclerosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21003-02 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Disposition of Halogenated Dibenzofurans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS
Other:	Hazel B. Matthews	Research Chemist	TRTP NIEHS
	Yiannakis M. Ioannou	Staff Fellow	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

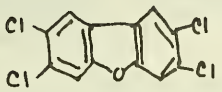
SUMMARY OF WORK (200 words or less - underline keywords)

Halogenated dibenzofurans are found worldwide as environmental pollutants. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the degree and position of halogenation. This work has established that 2,3,7,8-tetrachlorodibenzofuran (TCDF), an extremely toxic isomer, is excreted only after metabolism and toxicity is inversely related to metabolic capability. Thus, rats and mice, which readily metabolize TCDF, are relatively insensitive to its toxic action while guinea pigs are exquisitely sensitive and fail to metabolize this compound. The concept of a threshold body burden for toxicity is currently being tested. The absorption, distribution, metabolism and excretion of 1,2,4,6,8,9-hexachlorodibenzofuran (HCDF) has been investigated in rats. The nature of the metabolites produced from HCDF and TCDF is being examined.

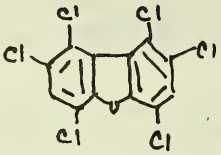
PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has used radioactively labeled compounds to quantitate absorption, distribution, metabolism and excretion of several polyhalogenated dibenzofurans (PHDFs). TCDF was labeled with ^{14}C; 1,2,4,6,8,9-HCDF was labeled with ^{3}H. The disposition of TCDF has been studied in two strains of mice after acute exposure and after repeated exposure in guinea pigs. HCDF has been studied in rats.



TCDF



HCDF

Analysis were facilitated by the use of a biological material's oxidizer and liquid scintillation counter. Metabolites are being purified and analyzed by thin layer chromatography and high pressure liquid chromatography. All data is subjected to further analysis by computer.

MAJOR FINDINGS AND PROPOSED COURSE:

The disposition of TCDF in two mouse strains was affected by their differential content of adipose tissue. C57BL/6J mice had approximately 1/2 as much fat as DBA/2J mice and half the whole body half-life. The greater amount of adipose tissues in DBA mice served to retain the TCDF and thus decrease its availability for metabolism and excretion. The enhanced size of the fat depot in these animals may in part explain the lessened sensitivity of this strain to the toxicity of halogenated polyaromatics.

Analysis of TCDF metabolites from rat bile has been initiated by preparing derivatives with trimethylsilane followed by purification on high pressure liquid chromatography.

Little metabolism of TCDF can be detected in the guinea pigs. It apparently stores TCDF in the fat until intoxication occurs. Then the fat is mobilized as an energy source and the TCDF is redistributed back to the liver. Upon repeated exposure to low levels of TCDF, no TCDF intoxication occurs until a critical body burden is reached. When this occurs, extensive weight loss and death ensue rapidly. The repeat dose studies indicate that the fat content affects the amount of TCDF needed to reach toxicity. Mature animals have larger fat deposits and have a higher LD_{50} than observed for young animals. The time to death also seems to increase with age while a lower percentage of the body weight seems to be lost before death occurs.

The disposition of ^{3}H-HCDF has been examined in order to extend the structure/activity relationships for polyhalogenated aromatics previously elucidated by members of this research group, specifically, Dr. H. B. Matthews. HCDF is

incompletely absorbed for the gut, but that portion of the dose which is absorbed was quite persistent, tending to accumulate more in the liver than in the fat. Small amounts of metabolites are excreted in the feces, but overall metabolism is minimal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated dibenzofurans are among the most toxic and persistent contaminants in the environment. Some of these chemicals have been implicated in human disorders. We need to be able to predict what the fate of these compounds will be in man. By the better understanding of their disposition in animals, we will be more able to estimate the risk to mankind.

PUBLICATIONS

Decad, G. M., Birnbaum, L. S., and Matthews, H. B.: Disposition of 2,3,7,8-tetrachlorodibenzofuran in guinea pigs, rats, and monkeys. In Hutzinger, O., Merian, E., and Frei, R. W. (Eds.): Impact of Chlorinated Dioxins and Related Compounds on the Environment. Oxford, Pergamon Press Ltd., 1982, pp. 307-315.

Matthews, H. B. and Birnbaum, L. S.: Factors affecting the disposition and persistence of halogenated furans and dioxins. In. Symposium Proceedings: International Symposium on the Chlorinated Dioxins and Related Compounds, Arlington, VA, 1981. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21004-02 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Senescent Changes in Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS
Other:	Michael Dieter	Research Physiologist	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.5	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age-related changes in many physiological parameters have long been known to occur. The basis for these alterations is, however, not well understood. Response to various stresses seems to decline with age. Changes in the ability to metabolize exogenous as well as endogenous compounds has been suggested as a cause for altered functions. This work will explore senescent changes in metabolism of several tissues--liver, lung, kidney, brain, lymphoid tissues. Enzyme systems such as glucuronyl transferase, β-glucuronidase, and those involved in intermediary metabolism and immune responses will be investigated. Altered disposition of chemicals in aging animals is being studied in order to elucidate the basis for age-related changes in pharmacological responses.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

This analysis of age-related changes in metabolism can basically be divided into two major divisions, altered pharmacological properties related to the body's ability to handle various drugs and environmental chemicals and alterations in intermediary metabolism in lymphoid tissues.

METHODS EMPLOYED: A colony of aging male Fischer F344 rats has been established by NIEHS at Charles River Laboratories. Weanling male rats, approximately 15 each month, are placed in the colony to be held until needed. An interium colony of retired breeder F344 male rats has been maintained at NIEHS. Animals available to us thus range in age from 1 through 30 months of age.

For studies of age-related changes, old animals (>24 months) will be compared to young adult animals (2½-6 mos) and to middle-aged ones (12-16 mos). If necessary, additional ages will be used.

MAJOR FINDINGS AND PROPOSED COURSE:

a) Altered pharmacological responses - Previous investigations by the principal investigator have involved an analysis of age-related changes in hepatic drug metabolism in vitro, specifically alterations in the mixed-function oxidases system. Currently, work is in progress to explore the effects of aging on glucuronyl transferase and β-glucuronidase activity in liver, lung, kidney, and small intestines.

Changes in the disposition of two polychlorinated biphenyls (PCBs) in aging rats have also been examined. 2,3,6,2',3',6'-hexachlorobiphenyl(236) and 2,4,5,2',4',5'-hexachlorobiphenyl(245) are symmetrical isomers whose distribution, metabolism, and excretion have been previously studied in young rats in our laboratory (Matthews and Tuey, 1980, Toxicol. Appl. Pharmacol. 53:377). In the present study, senescent rats (22-24 months) were treated iv from 1 hr to 21 days and distribution of a ^{14}C-labeled dose (0.6 mg/kg) was examined in tissues and excreta. The half-lives and pool sizes were increased for both compounds, suggestiong a slower rate of metabolism. Increased metabolite/parent ratios in the tissues suggested a slower elimination of metabolites in the old rats. The effects of aging seems to be more pronounced for 245 than for 236. Thus senescence may differentially affect the disposition of more persistent PCBs.

b) Alterations in intermediary metabolism in lymphoid tissue - Lymphatic tissues and associated cells, including thymus, spleen, and macrophages, were examined for age-related changes in intermediary metabolism. Key enzymes from the hexose monophosphate shunt, glycolysis, and the Krebs cycle were compared in rats of 6,12,18, and 24 months of age. Pyruvate kinase and lactate dehydrogenase decreased in thymus, but increased in spleen. Glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase activities all decreased in pulmonary macrophages. These data suggest that the biochemical support for phagocytosis and cell-mediated immunity are diminished during aging in macrophages and in the thymus, while the humoral immune response mediated by splenic B cells may not be compromised in senescent rats.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21005-02 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Metabolism and Disposition of Aromatic Amines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	TRTP NIEHS
	Yiannakis M. Ioannou	Staff Fellow	TRTP NIEHS
	Harish Chopade	Visiting Fellow	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	1.6	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the absorption, distribution, metabolism and excretion of a series of four ^{14}C-labeled aromatic amines, p-nitroaniline, diphenyl guanidine, 2,4-dinitroaniline, and 4-chloro-2-nitroaniline in the rat indicate that clearance rates vary with compound, but that each compound is readily absorbed, rapidly metabolized and excreted in the form of several metabolites. Excretion of most parent compounds prior to metabolism is minimal. These compounds do not appear to be accumulated to a significant extent in any tissue, possibly due to the fact that they are rapidly metabolized. Within a few hours after administration most of the dose retained by the body was in the form of metabolites. These compounds were eliminated in both urine and feces, and whole body half-lives for each compound was less than 1 day. Additional studies of these and other aromatic or heterocyclic amines will provide a better understanding of the importance of chemical structure to metabolism, disposition and persistence of this class of compounds.

PROJECT DESCRIPTION

METHODS EMPLOYED: The absorption, distribution, metabolism, and excretion of a series of aromatic amines is being studied following oral and iv administration to adult male rats. Absorption and distribution to the major tissues and clearance from the tissues into excreta are quantitated by utilizing ^{14}C-labeled compounds, serial sacrifice, sampling and oxidation of samples to $^{14}CO_2$ in a biological material oxidizer for analysis by liquid scintillation counting. Metabolite identification involves solvent extraction of tissues and excreta, purification by thin-layer and high pressure liquid chromatography and co-chromatography with authenic standards. Kinetic parameters are based on the disposition data and are calculated by computer.

MAJOR FINDINGS AND PROPOSED COURSE:

1) 1,3-diphenylguanidine (DPG), a rubber accelerator, was readily absorbed from the gastrointestinal tract of the male Fischer rat and rapidly distributed throughout the body tissues. Absorption and disposition of DPG were not significantly effected by the route of administration or by the dose in the 1.5 to 150 μmol/kg dose range studied. Most of the dose of DPG was excreted in the urine and feces at approximately equal amounts within 24 hr after oral or iv administration. Greater than 99% of the DPG dose was cleared into the urine and feces within 3 days after administration. Approximately 50-60% of the DPG derived radioactivity excreted in urine was the parent compound, DPG, while the remainder was present in the form of one major and two minor metabolites. Close to 90% of the radioactivity excreted in bile was in the form of a single major metabolite. Administration of multiple doses resulted in a proportional increase of DPG derived radioactivity in the liver as the number of doses increased. DPG derived radioactivity did not accumulate in other tissues follwoing either single or multiple doses.

2) p-Nitroaniline was readily absorbed from the gastrointestinal tract distributed to all the tissues by blood and rapidly excreted. p-Nitroaniline was excreted in urine in the form of a number of metabolites. The half-life for clearance from most tissues was less than 12 hours and little p-nitroaniline derived radioactivity remained in the body after 24 hours. Trace levels of p-nitroaniline were most persistent in blood, possibly due to a reaction with hemoglobin. Metabolites of p-nitroaniline were excreted as conjugates, primarily sulphate conjugates, in the urine and bile. Excretion of unmetabolized parent compound accounted for a relatively small part of the dose.

3) Studies of the absorption, distribution and excretion of 4-chloro-2-nitroaniline (CNA) indicate thus this chemical was near completely absorbed from the gastrointestinal tract after oral doses of 0.136, 1.36, and 13.6 mg/kg body wt. The distribution pattern was the same whether treatement was by oral or intravenous administration. The adipose tissue was the major depot of CNA, with moderate amounts being redistributed to the skin. CNA was primarily excreted in the urine. More than 65% was excreted in the urine in 7 hours. About 12% was excreted via the bile in the feces within 3 days. CNA-derived radioactivity excreted in the urine over 3 days was approximately 75%. Studies of CNA metabolism indicated that this chemical is readily metabolized to at least

nine metabolites. The major metabolite has been identified as a sulfate conjugate of the parent compound. This metabolite accounts for 65% of the total dose and is excreted almost exclusively in urine. The minor metabolites have not been identified.

4) The disposition of 2,4-dinitroaniline (DNA) was independent of dose or route of administration when adminstered orally at doses of 1, 10 or 90 μmol/kg or by iv injection at 10 μmol/kg. Absorption from the gastrointestinal tract was near complete throughout the range of oral doses. Total excretion was predominantly in urine (70%) and accounted for approximately 90% of the dose within 24 hr. Most of the DNA derived material excreted was in the form of conjugates of various metabolites. There was no evidence to indicate that the parent compound or any of its metabolites was retained in any of the fifteen major tissues assayed.

PROPOSED COURSE: The metabolism and disposition of at least one more aromatic amine, 2-bromo-4,6-dinitroaniline, and a series of heterocyclic amines will be studied in the rat. In these studies there will be a special emphasis on the isolation and identification of metabolites which are known or suspect carcinogens. Additional goals of these studies will be to observe the effect of halogenation, nitro groups and polarity on metabolism, disposition and bioaccumulation. Further work on aromatic amines will be planned according to the results of these studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aromatic amines are widely used as industrial chemicals, particularly in the rubber and dye industries. Annual use of individual chemicals frequently exceeds one million pounds per year and as a group these chemicals account for many millions of pounds per year. A number of aromatic amines, such as β-naphthalamine, benzidine and 4-aminobiphenyl are known human carcinogens and measures have been taken to remove these chemicals from the market and to minimize industrial exposure. However, the toxicology of the aromatic amines which are currently in use is largely unknown. It is known that on acute exposure some aromatic amines are quite toxic whereas others are relatively innocuous; however, little is known about the metabolism, disposition or persistence of these chemicals and knowledge of their potential for chronic toxicity is almost completely lacking. Some aromatic amines have structures which suggest that they might be metabolized to known carcinogens or closely related compounds. The present work is designed to isolate and identify metabolites of these chemicals and determine how various groups on the aromatic rings affect absorption, metabolism and disposition of aromatic amines. These studies may identify potentially carcinogenic aromatic amines which are currently in industrial use and should provide a better understanding of those chemical structural factors which facilitate or inhibit absorption, metabolism and elimination of these compounds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21009-02 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Reproductive Effects in Males Exposed to Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.C. Lamb	Research Biologist	STB NIEHS
Others:	R.E. Chapin	Staff Fellow	STB NIEHS
	J.A. Moore	Deputy Director, NTP	STB NIEHS
	E.E. McConnell	Chief, CPB	CPB NIEHS
	W.M. Kluwe	Pharmacologist	CTEB NIEHS

COOPERATING UNITS (if any)
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Data Management and Analysis
Carcinogenesis and Toxicology Evaluation Branch

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Fertility and Reproduction Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various environmental and industrial chemicals can disturb male reproductive function. The objective of these studies is to enhance our understanding of that toxic potential, and to further elucidate the mechanism of action in chemicals found to be toxic. Chemicals which are active as chemosterilants in males, such as dibromochloropropane and the phthalate esters, are used in various test systems. In addition to mechanistic studies, chemicals of unknown activity, such as the phenoxy herbicides and TCDD, have also been studied. Endpoints of toxicity include the assessment of spermatogenesis, sperm morphology, and hormone levels. Studies are beginning on the morphological response of the testis to chemical exposure. Androgen Binding Protein (ABP) assays will also be performed to assess Sertoli cell function. These studies are expected to yield valuable data on chemical toxicity in males, as well as improve the sensitivity and accuracy of future testing systems.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: In addition to histological evaluation of testes and accessory sex organs, these studies involve assessment of sperm head morphology from the cauda epididymis and vas deferens. The treated males have been studied by fertility and mating experiments and hormone patterns were studied in treated and control animals. Special high resolution light microscopic studies of the testis have been initiated. Testicular function will also be evaluated by measuring ABP secretion by the Sertoli cells.

MAJOR FINDINGS AND PROPOSED COURSE: Studies have been completed on the effects of DBCP on the fertility of male rats. A wide spectrum of fertility endpoints were used in those studies and the recovery of the testis after DBCP exposure is now in progress.

Subsequent studies are in progress which further investigate male germ cell toxicity as it related to fertility and testicular function using other model compounds which affect male reproductive function.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The important potential of chemicals to alter fertility and reproductive function is just beginning to receive appropriate attention. Studies are anticipated or are in progress using known mutagens and/or chemosterilants which will expand our knowledge of these chemicals' toxic mechanisms. Such information will allow us to develop more predictive test systems in this field.

PUBLICATIONS

Lamb, J.C., IV, Marks, T.A., McConnell, E.E., Abeywickrama, K., and Moore, J.A.: Toxicity of chlorinated phenoxy acids in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6 male mice. J. Tox. Environ. Hlth. 8: 815-824, 1981.

Lamb, J.C., IV, Marks, T.A., Gladen, B.C., Allen, J.W. and Moore, J.A.: Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Tox. Environ. Hlth. 8: 825-834, 1981.

Lamb, J.C., IV, Marks, T.A., Haseman, J.K., and Moore, J.A.: Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Tox. Environ. Hlth. 8: 835-844, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21024-01 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Chemicals on Drug-Metabolizing Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Goldstein	Pharmacologist	TRTP NIEHS
OTHER:	D. Sundheimer	Staff Fellow	TRTP NIEHS
	P. Linko	Chemist	TRTP NIEHS

COOPERATING UNITS (if any)

M. I. Luster, STB, NTP
Ian Robertson, CGTB, NTP

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this study are to identify and characterize changes in subspecies of cytochrome P-450 which occur in the rat after treatment with polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD) and other environmental chemicals and to assess the implications of these changes. Present work includes purification and characterization of a subspecies of cytochrome P-450 from livers of rats treated with 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) and comparison with P-450$_{PB}$ and P-450$_{MC}$ isolated from phenobarbital and 3-methylcholanthrene (3-MC) treated rats. The cytochrome from HCB treated rats differs from the other two in its molecular weight, peptide maps, immunological properties and catalytic activities. This cytochrome is induced by all 3-methylcholanthrene type inducers tested. An RIA has been developed for quantitation of these cytochromes in tissues.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS: 1) Three subspecies of cytochrome P-450 have been purified (10-19 nmol/mg) from livers of 3-MC, HCB and phenobarbital treated rats. Cytochrome P-450$_{HCB}$ differs from the major cytochrome isolated from phenobarbital 3-MC treated rats in its immunochemical properties, molecular weight (52,000), peptide maps, and spectral properties. Its oxidized spectrum peaks at 392nm (high spin) and its CO-reduced at 448 nm. Ouchterlony double-diffusion plates indicate the three proteins are immunologically distinct.

2) The catalytic activity of the three enzymes have been compared after reconstitution with cytochrome c reductase and lipid. Catalytically P-450$_{HCB}$ differs from P-450$_{PB}$ and P-450$_{MC}$ in that it has low catalytic activity toward benzphetamine, benzo[a]pyrene (aryl hydrocarbon hydroxylase) or ethoxyresorufin. However, it has high catalytic activity toward the precarcinogen acetylaminofluorine (AAF). Both P-450$_{MC}$ and P-450$_{HCB}$ metabolize AAF, but P-450$_{HCB}$ produces more of the mutagenic N-OH metabolite. P-450$_{HCB}$ also has high activity toward amines such as acetanilide and aniline. Km's and Vmax's for each substrate have been estimated.

3) Specific antibodies have been developed for P-450$_{MC}$ and P-450$_{HCB}$ by the use of immunoabsorbants. An RIA has been developed which shows less than 0.50% cross reactivity. This RIA indicates that all 3-MC type inducers tested (3-MC, 3,4,5,3',4',5'-HCB and TCDD) induce both P-450$_{MC}$ and P-450$_{HCB}$ 50-100 fold. Neither cytochrome is a major constitutive enzyme.

3) The monospecific antibodies have been titrated against each of reconstituted catalytic activities. Anti-P-450$_{MC}$ is very active, against the catalytic site.

4) Mutagenesis experiments have been initiated. P-450$_{HCB}$ is more active than the other two P-450s toward AF (N-hydroxylation). Activity toward other precarcinogens is underway.

PROPOSED COURSE:

Antibody experiments with whole liver microsomes will be used to identify which pathways are mediated by the respective enzymes. Mutagenesis experiments against additional substrates are in progress. RIA experiments will be used to determine whether environmental chemicals affect these enzymes in extrahepatic tissues and in the embryo. The immunoprecipitation and in vitro translation of the 3-MC inducible messages will be examined to determine whether these messages are expressed co-ordinately. We will examine and attempt to isolate additional enzymes including the constituitive enzymes from control animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OFTHE INSTITUTE: The P-450 system is the principle enzyme system which catalyzes foreign chemicals including carcinogens to the ultimate labile carcinogen. Many endogenous compounds (lipids and hormones) are also metabolized by this electron transport system. Present investigations are particularly oriented toward changes in the metabolism of potential carcinogens and mutagens after exposure to environmental chemicals. Changes in lipid metabolism will also be investigated and may be important in PCB and TCDD induced toxicity.

PUBLICATION

Goldstein, J. A., Linko, P., Luster, M. I. and Sundheimer, D. W.: Purification and characterization of a second form of hepatic cytochrome P-448 from rats treated with a pure polychlorinated biphenyl isomer. J. Biol. Chem. 257: 2702-2707 (1982).

Goldstein, J. A., Linko, P., McKinney, J. D., and Albro, P. W.: Marked differences in the inductive effects of two symmetrical hexachlorobiphenyls and the corresponding unsymmetrical isomer on hepatic monooxygenases, Biochem. Pharmacol., 30: 1008-1011, 1981.

Kohli, K. K., Linko, P. and Goldstein, J. A.: Multiple forms of solubilized and partially resolved cytochrome P-450 from rats induced by 2,3,5,2',3',5'- and 3,4,5,3',4',5'-hexachlorobiphenyls. Biochem. Biophys. Res. Commun., 100: 483-490, 1981.

Kohli, K. and Goldstein, J. A.: Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic and renal prostaglandin synthetase, Life Sciences, 29: 299-305, 1981.

Goldstein, J. A., Linko, P., Huckins, J. N. and Stalling, D. L.: Structure-activity relationships of chlorinated benzene as inducers of multiple forms of cytochrome P-450 in rat liver. Chem.-Biol. Interact., 1982, In Press.

Goldstein, J. A., Linko, P. and Bergman, H.: Induction of porphyria in the rat by subchronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Pharmacol., 1982, In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21025-01 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Disposition of Santonox

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX(c) NEITHER

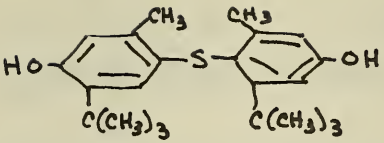
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Santonox, 4,4'-thio-bis(6-tert-butyl-m-cresol), has been recommended for study in the NTP as a representative of the class of rubber antioxidants which have widespread industrial usage and a high potential for occupational exposure. Santonox is relatively non-toxic, with an oral LD_{50} of approximately 5g/kg. Before being tested in the bioassay program, disposition studies are needed to assess its absorption, distribution, metabolism, and excretion. Such studies will not only result in more appropriate dose settings, but a better understanding of the mechanism of toxicity of this class of compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION



Santonox

METHODS EMPLOYED: ^{14}C-labeled Santonox will be used in order to study the chemical disposition of this antioxidant. Absorption from the gastrointestinal tract will be determined at 3 doses, the highest being 1/10th of the oral LD_{50}. The distribution after an iv dose will be examined at varying time points after treatment. Excreta and expired air will be examined for radioactivity by oxidation and trapping of $^{14}CO_2$ and liquid scintillation counting. The radioactivity will be resolved into parent compound and metabolites by organic solvent extraction and thin layer chromatography and/or high pressure liquid chromatography. Metabolite characterization will also be carried out by chemical and enzymatic means.

SIGNIFICANCE: This compound is of unknown suspicion of carcinogenicity but is considered to have high potential for human exposure based on its production. It is a representative of the eight sulfides and disulfides included in the rubber processing chemicals class study. Analysis of the disposition of Santonox should relieve the gap in the knowledge of such compounds which currently exists.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21026-01 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Disposition of Hexabromonaphthalene

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.2	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

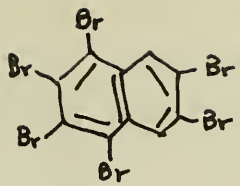
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bromonapthalenes have no known industrial use or application, but have been identified as contaminants of Firemaster BP-6, the toxic mixture of polybrominated biphenyls used as a fire retardant and involved in a major episode of environmental posioning in Michigan. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the position of bromination. This work has studied the chemical disposition of 1,2,3,4,6,7-hexabromonapthalene (HBN), the most toxic bromonaphthalene in rats. The compound is incompletely absorbed after an oral dose. After iv treatment nearly 50% of the dose is excreted as metabolites within 3 days. However, the remainder of the dose seems to be extremely persistent, over 25% remaining in the liver after 35 days. It is possible that the compound spontaneously rearranged to its less toxic isomer, 1,2,3,5,6,7-HBN, which is also more resistant to oxidation. This suggests that what is being observed is the rapid metabolism and excretion of 1,2,3,4,6,7-HBN, while the rearrangement product persists.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION



HBN

METHODS EMPLOYED: This work has used ^{14}C-labeled compound to quantitate the chemical disposition of 1,2,3,4,6,7-HBN in male Fischer 344 rats after acute oral and iv exposure. Analyses were facilitated by the use of a biological materials oxidizer and liquid scintillation counting. Tissue extraction with organic solvents was followed by thin layer chromatography in order to resolve parent compound from metabolites.

MAJOR FINDINGS: HBN is incompletely absorbed from the gut. It initially concentrates in the liver and some of the compound is rapidly metabolized in the liver and excreted via the bile into the feces. The remainder is rapidly distributed to the fat and skin from which it slowly returns to the liver where it persists. During the first few days after acute exposure, metabolites were excreted in the feces. Almost no radioactivity appeared in the urine. Following the initial burst of excretion, little HBN-derived radioactivity was excreted from 3 to 35 days. This agrees with the persistence of the remainder of the dose in the liver. The basis of the rapid initial metabolism followed by the remainder being persistent is being investigated.

SIGNIFICANCE: It has been estimated that brominated napthalenes may account for 20% of the toxicity of the Firemaster BP-6 mixture, and thus may be involved in the human and domestic animal toxicity observed in Michigan. The disposition of HBN relative to that of other halogenated aromatic compounds provides further understanding of the disposition of this broad class of compounds in the environment and better enables us to predict their risk to man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21027-01 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

"Metabolism and Disposition of Allyl Isothiocyanate in Rats"

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yiannakis M. Ioannou	Staff Fellow	TRTP NIEHS
	H. B. Matthews	Research Chemist	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The absorption, distribution, metabolism and excretion of ^{14}C labeled allyl isothiocyanate (AITC) was studied in male and female F344 rats. AITC is readily absorbed, metabolized and excreted in the form of several metabolites. Approximately 60-70% of the dose is excreted in urine within 24 hr in both male and female rats following either oral or iv administration of AITC. Only 2-3% of the dose is excreted in feces. The whole body half-life for AITC was less than 24 hr in both sexes. High concentration of AITC derived radioactivity was present in blood and lung 15 min after administration and in liver, muscle, skin and adipose tissue 45 min after administration. Very small amounts of AITC were present 24 hr after administration. This compound appears to have little or no potential for bioaccumulation in any tissue assayed.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The absorption distribution metabolism and excretion of AITC was studied following oral and iv administration to adult male and female F344 rats. Absorption and distribution to the major tissues and clearance from the tissues into excretia were quantitated by utilizing ^{14}C-labeled AITC, serial sacrifice, sampling and oxidation of samples to $^{14}CO_2$ and quantitation of radioactivity by liquid scintillation counting. Major tissues, urine and feces were extracted by different solvents and the metabolites present were analysed and quantitated by HPLC. Kinetic parameters were based on the disposition data and were calculated by computer.

MAJOR FINDINGS: AITC was readily absorbed from the gastrointestinal tract, distributed to all tissues and rapidly excreted in the urine in the form of several metabolites. The half life for clearance from most tissues was less than 6 hr and little (less than 5%) of AITC derived radioactivity remained in the body after 24 hr. AITC does not appear to bioaccumulate in any particular tissue. Radioactivity extracted from major tissues was primarily in the form of 2 major metabolites with only traces of parent compound present at all time points examined. The metabolites formed by male and female rats were qualitatively similar, but showed quantitative variations in the two sexes depending on the tissues and time of sacrifice. AITC-treated rats voided a significantly greater volume of urine than the controls and female rats excreted more than twice as much urine as did male rats on a per kilogram body weight basis.

PROPOSED COURSE: The metabolism and disposition of AITC will also be studied in male and female B6C3F1 mice. The results of this study will be compared to those obtained from male and female F344 rats in an attempt to explain differences in AITC induced urinary bladder tumor formation between sexes and species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: AITC is a common food additive which has recently been shown to be carcinogenic to male F344 rats causing transitional cell papillomas of the urinary bladder. Since AITC is present in foods the potential for human exposure is very high. However, little is known about the metabolism and disposition or mechanism of action of this chemical. The present study is designed to investigate differences in metabolism and disposition of AITC between male and female F344 rats and male and female B6C3F1 mice. The results of this study will prove useful in the evaluation and interpretation of the NTP Bioassay Report and help determine if the carcinogenic activity of this compound is unique to the male rat.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 21029-01 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Kepone Toxicity as a Model for an Environmental Chemical's Influence on Female Reproduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.C. Lamb, IV	Research Biologist	STB NIEHS
Others:	J.A. Moore	Deputy Director, NTP	TRTP NIEHS
	E.E. McConnell	Chief, CPB	CPB NIEHS
	K.S. Korach	Research Endocrinologist	LRDT NIEHS
	J.S. Hong	Pharmacologist	LBNT NIEHS

COOPERATING UNITS (if any)
Chemical Pathology Branch
Laboratory of Reproductive and Developmental Toxicology
Laboratory of Behavioral and Neurologic Toxicology

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Fertility and Reproduction Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.55	0.95

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of these studies is to evaluate the potential of environmental chemicals to affect female reproductive function. These studies compare toxic effects at high and low levels of exposure. As a model compound for these experiments, we are studying the effects of Kepone on female reproductive function. The toxicity of these compounds is evaluated using a broad spectrum of toxic indicators. Since these effects, and the effects of other environmental compounds, may be mediated through their estrogenic or other hormonal activity, we have established a number of criteria which indicate hormone activity. Uterine, ovarian and pituitary function are studied in morphological and endocrinological studies after Kepone exposure. Fertility and reproduction are also evaluated in treated females and in offspring exposed perinatally to Kepone. Morphological studies include light and scanning electron microscopy, hormone and xenobiotic autoradiography, and histochemistry. Biochemical studies include hormone radioimmunoassay and hormone receptor assays. These studies will help establish the mechanism of reproductive toxicity of compounds such as Kepone and should lead to more efficient and accurate testing systems in reproductive toxicology.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Initial studies on the reproductive toxicity of chemicals have involved the assessment of fertility. These investigations included the long-term chemical exposure of female rats and mating to untreated males. Chemical distribution studies have demonstrated the bioaccumulation of Kepone, and have been used to evaluate various chemical delivery systems. A new dosing system was used to compare the ability of chlordecone to diethylstilbestrol to cause pituitary tumors in F344 rats. This system involved adding the chemicals to silastic tubing implants which were implanted subcutaneously. A pituitary cell culture system has been established to determine how the pituitary responds in vitro to xenobiotics, such as Kepone and DES. Kepone is one of a number of compounds identified as an environmental estrogen.

The autoradiographic subcellular localization of steroid hormones has been studied. The localization of ^{3}H-estradiol in the uterus is of special interest, since Kepone may act through the same receptors as estradiol and the localization of estradiol will be compared to radiolabeled-Kepone localization. These morphological studies of hormone-receptor action are being correlated with biochemical hormone receptor studies on estradiol and Kepone.

MAJOR FINDINGS AND PROPOSED COURSE: Studies in which Kepone was added to the diet showed that even through Kepone did accumulate in body tissues, including the uterus, it could be given at levels which did not disturb normal reproductive function. At higher exposure levels, rats exhibited an estrogen response to Kepone. Since estrogens cause pituitary tumors in a short time in F344 rats, we compared the tumorigenicity of Kepone to DES, a potent estrogen. We found that the endocrine and pituitary toxicity of Kepone was much lower than expected at maximal exposure levels. Pituitary cell culture studies are underway to evaluate the neuroendocrine toxicity of Kepone. Further studies on the subcellular localization of Kepone in the rat uterus and pituitary are also anticipated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The knowledge gained on the endocrine and reproductive toxicity of Kepone should increase our understanding of normal endocrine function and the response of the reproductive system to xenobiotics. These studies fulfill two major functions within the biomedical research community. They expand our understanding of the role which environmentally relevant compounds may play in affecting reproductive function, and they help develop a much needed basis for the design of reproductive toxicology testing systems.

PUBLICATIONS

Korach, K.S. and Lamb, J.C., IV.: Estrogen action in the mouse uterus: differential nuclear localization of estradiol in uterine cell types. Endocrinology 108: 1989-1991, 1981.

Ali, S.F., Hong, J.-S., Lamb, J.C., Moore, J.A., and Bondy, S.C.: Subchronic dietary exposure to rats to chlordecone (Kepone^{R}) modifies levels of hypothalamic β-endorphin. Neurotoxicology, in press, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES30044-06 STB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Toxicology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Van Stee	Physiologist	STB NIEHS
OTHER:	J.E. Simmons	Graduate Student	STB NIEHS
	R.A. Sloane	Biological Laboratory Technician	STB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.1	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Rats were exposed by inhalation to 7 different concentration profiles of carbon tetrachloride. Each profile had a total concentration times time equal to 4500 ppm-hr. Some of the profiles could be distinguished from others based on histopathological changes in the livers. The toxic response was not consistently related to number of, duration of, or interval between pulses.
2. and 3. Studies are underway to determine the mechanism of the acceleration of atherosclerosis by carbon disulfide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Male, CD rats were exposed in groups of 12, to 7 different exposure profiles of carbon tetrachloride (CCl4) in which the maximum concentration was 1500 ppm. The exposures were conducted in a computer-assisted inhalation facility in dynamic flow-through chambers of a capacity of 400 liters. The flow rate was 100 liters/min. The exposures consisted of sequences of approximately rectangular pulses of varying duration and interval. The total concentration-times-time (CxT) for each profile was 4500 ppm/hr. The rats were exposed daily for 4 consecutive days and killed on the 5th day. Sections of livers from each of 3 rats selected at random from each exposure group of 12 were examined microscopically. Features of necrosis and vacuolation were recorded as either present or absent and severity was quantified on a scale of 0 through 4. Experiments were replicated at least 6 times. Ranks representing severity were transformed to normal scores. All data were analyzed using the analysis of variance with Duncan's New Multiple Range Test, and multiple linear regression analysis. 2. Male CD rats and NZW rabbits were exposed to carbon disulfide (CS2), 300 ppm, 6 hr/da, 5 da/wk for 12 wk in the facility described in #1. The animals were weighed and thyroid function tests were performed every 2 wks. 125-I uptake was determined by gamma counting and serum activities of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) were done by radioimmunoassay (RIA). Data were analyzed using the analysis of variance and regression analysis. 3. Twenty-seven male, NZW rabbits were divided into groups of three and exposed to all possible combinations of 0, 1, or 2% dietary cholesterol, and 0, 100, or 300 ppm of CS2, respectively, for 12 wk. Changes in serum and aortic lipids were analyzed as indexes of the atherogenic process. Fat-stained, frozen sections of arteries, and sections stained with H&E following standard fixation were examined microscopically. Data were analyzed using the analysis of variance and regression analysis.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Some of the CCl4 exposure profiles could be distinguished from others by the histopathological changes that were observed in the liver. Hepatotoxicity was not significantly related to number of leading edges (number of pulses), duration of pulses, or the interval between the pulses. Either we haven't been able to extract the information or we don't have enough information to decide what characteristics of the profiles are responsible for the differential responses. A better model than ours could be chosen for the purpose of investigating the dependency of an expression of toxicity on exposure profile. The model should exhibit a readily quantifiable dose-response, and interpretation of the endpoints should not be confused by complicated pharmacokinetics. A chemical not requiring activation and acting as a primary pulmonary toxin might be suitable. 2. Data from this experiment were not available at the time of the writing of this report. If a depression of thyroid activity can be correlated with an acceleration of atherogenesis in this and other experiments, future experiments will be conducted in which animals will be exposed to an atherogenic diet and CS2, and fed thyroid hormones to see if thyroid replacement therapy mitigates this effect of exposure to CS2. 3. Data from this experiment were not available at the time of the writing of this report. If a fraction of the atherogenic effect of exposure to CS2 is found not to be simply a secondary response to hyperlipidemia experiments will

be conducted to examine the possibility that CS2 induces intimal injury that could trigger the atherogenic cascade described by the "injury hypothesis" of atherogenesis. 4. If the resources become available CS2 will be screened using the Strain A/J mouse, carcinogen bioassay system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1. We have concluded that under certain circumstances the shape of the concentration profile to which animals are exposed may affect the outcome of experiments in inhalation toxicology. The time-weighted average concentration may not always represent the best model of time-varying, real-life conditions. Few comparisons of different exposure profiles with equal time-weighted concentrations have been reported in the past because of the technical difficulty in simulating the conditions in the laboratory. Computer-assisted operation of inhalation facilities eliminates this impediment to research. Exposure profiles monitored in the real world may now be simulated easily and reproducibly thus paving the way for ruling in or out the significance of this generally ignored variable. 2. Observers estimate that between 20,000 and 500,000 (depending on who is doing the reporting) Americans are exposed to CS2. CS2 has a multiplicity of toxic effects and, despite decades of research, many areas remain relatively unexplored. The National Toxicology Program has selected CS2 for further study, and the results of our efforts are expected to clarify the matter of whether or not the depression of thyroid activity that is induced by exposure to CS2 contributes to the acceleration of the development of atherosclerosis that also accompanies long-term, low-level exposure to CS2. If hypothyroidism turns out to be of clinical significance, and exposure to CS2 cannot be avoided, our studies may indicate that it could be possible to mitigate the effects by the appropriate therapy.

PUBLICATIONS

Van Stee, E.W., Wynns, P.C., and Moorman, M.P.: Distribution and disposition of morpholine in the rabbit. Toxicology 20: 53-60, 1981.

Van Stee, E.W., Boorman, G.A. and Moorman, M.P.: Time-varying concentration profile as a determinant of the inhalation toxicity of CCl4. J. Toxicol. Environ. Health (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER * Z01 ES 30106-03 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Effects of Environmental Pollutants on the Immune System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.I. Luster	Research Microbiologist	STB NIEHS
Other:	G.A. Boorman	Veterinary Medical Officer	CPB NIEHS
	M.P. Dieter	Physiologist	CTEB NIEHS

*Continuation of 4 projects for LEC: Z01 ES 10005-02, ES 10006-02, ES 30062-4, ES 10010-01

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Immunotoxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.4	1.4	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The on-going objectives of the immunotoxicology group are to select, refine and validate a panel of immune and host resistance procedures to better define immunotoxicity and correlate changes in immune function with altered host-resistance. We are completing the 2nd year of a two-year evaluation study examining a comprehensive panel of assays of which a number of these procedures have been selected for interlaboratory validation using suspect immunotoxic chemicals. This comprehensive assay panel examines: (1) host resistance to parasitic, bacterial, and tumor cell challenge; (2) cell-mediated immune functions; (3) natural killer (NK) cell activity; (4) bone marrow progenitor cell function; 5) humoral immune functions; and (6) macrophage function. We have examined selected chemicals of environmental concern as to their effect on the immune system and host resistance. Most of these chemicals are known human carcinogens and were examined to determine: (a) the carcinogenic-immunotoxic relationship; (b) structure-activity relationships; and/or (d) mechanisms of immunotoxicity. This approach should potentially allow for accurate assessment of human health risk as well as determining no-effect levels.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The assays that are being developed, validated or refined in a comprehensive panel to assess immunological dysfunction or altered host resistance following chemical exposure are listed in Table 1. A multiple assay panel is necessary because of the complexity of the immune system.

Table 1
Comprehensive Screening Panel for Defining Immune Alterations
Currently Being Evaluated at NIEHS

Parameter	Procedure Performed
Pathotoxicology	Hematology Profile-hemoglobin, red blood cell count, white blood cell count, differential
	Clinical Chemistries-CPK, αHBDH, SGTP, BUN, creatinine, acid and alkaline phosphatase, LDH, cholinesterase
	Serum Proteins-albumin, globulin, A/G, total proteins
	Weights-body, spleen, thymus, liver, kidney
	Histology-liver, thymus, adrenal, lung, kidney, heart, spleen
Host Resistance	Tumor Assays-tumor cell challenge TD_{10-20} and radiometric tumor mass
	Listeria monocytogenes LD_{10-20} challenge
	Pseudomonas aeruginosa LD_{10-20} challenge
	Plasmodium induced parasitemia
Delayed Hypersensitivity	Radiometric assay with T-cell dependent antigen
Lymphocyte Proliferation	One-way mixed leukocyte culture
	Mitogens-PHA, Con A, LPS
	Enzymes from the HMS, EM and TCA cycles
Humoral Immunity	Immunoglobulin levels (IgG, IgM, IgA)
	Antibody response to T-dependent (SRBC), T-independent (LPS), B1 (TNP-LPS) and B2 (TNP-Ficol) antigens
Macrophage Function[1]	Resident peritoneal cell numbers and differential
	Phagocytosis

	Lysosomal enzymes-5'-nucleotidase, acid phosphatase, leucine amino peptidase and HMS enzyme
	Cytostasis of tumor target cells
Bone Marrow Colony Forming Units	CFU-S-multipotent, hematopoietic stem cells
	CFU-GM-granulocyte/macrophage progenitor
	Cellularity

[1]Employs both resident peritoneal cells and BCG activated macrophages

MAJOR FINDINGS AND PROPOSED COURSE: An abbreviated version of the assay panel is being employed in the short term testing phase of NTP's chemical bioassay program to evaluate its utility for detecting the immunotoxicity of suspect chemicals and drugs. This protocol will only be performed in BOA laboratories who have demonstrated assay competence by the performance criteria specified in the protocol with approval by the Immunology Program.

Host resistance assay models have been expanded in FY-82 to include survival following challenge with Pseudomonas aeruginosa as well as parasitemia and anemia following challenge with a non-lethal strain of Plasmodium yoelii. These assays appear to correlate with macrophage function and humoral immunocompetence. The assays for T-cell immunocompetence including Listeria and tumor cell challenge are now validated. Capabilities in examining T-independent antibody responses have been expanded with the addition of the T-independent antigen TNP-LPS and TNP-Ficol which activate B1 (immature B-cells) and B2 (mature B-cells), independently.

Capabilities for detecting altered macrophage function have been further expanded through the development of an in vitro fluorescent phagocytosis assay using "luminol beads". The macrophage enzyme panel has been expanded to include ecto-enzyme (5'-nucleosidase).

Immunological studies with inhaled materials were initiated this year to examine both systemic and local (lung) immune functions evaluated. The effect of chrysotile asbestos exposure on immune and bone marrow parameters were examined in mice at 2, 12, and 26 weeks following a 3 day exposure. The number of pluripotent bone marrow stem cells (CFU-S) and marrow macrophage granulocyte progenitors were decreased at all three time periods. In general, immune alterations were not present in mice at 1 and 3 months following exposure but selected affects were found at 5 months. This correlated with histopathological findings where evidence of asbestos related centriacinar pulmonary fibrosis and histiocytosis was first found at 5 months. Immune alterations included elevated numbers and enhanced activity of alveolar macrophages as evidenced by increased phagocytosis and tumor cell cytostasis activity. As reported in humans, there was evidence for slightly increased numbers of antibody plaque forming cells. The lymphoproliferative response to the B-cell mitogen was also enhanced at 12 weeks. These studies demonstrate that inhalation of asbestos fibers can produce delayed systemic

immune alterations in mice. Further, the delayed onset of these immune alterations occur concurrently with morphological alterations in the lung that are characteristic of asbestos. It is felt that the mouse model may prove useful in characterizing the nature of immune dysfunction that has been reported in asbestos exposed patients and we plan to continue these studies examining long term (1 year) effects.

In other studies, B6C3F$_1$ mice were exposed to vinyl chloride (1000 ppm) for 30 days for 5 hours each day. Controls were exposed to air. Treated mice revealed liver pathology (accentuation of the hepatic lobular patterns) which was consistent with high dose vinyl chloride exposure. However, there were no changes in pathotoxicology parameters, immune function or host resistance. No further studies are planned with vinyl chloride.

In studies with heavy metals, B6C3F$_1$ male mice were given water containing 3, 15, and 75 ppm mercury (as mercuric chloride) for 7 weeks. There were dose-related increases in blood and kidney mercury levels but only the former showed a time-dependent change. Non-specific toxicity occurred at the 75 ppm dose level, consisting of small differences in body and organ weights, hematological changes, and general enzyme inhibition in the bone marrow and spleen. However, there were specific immunotoxic and biochemical alterations in lymphoid organs of mice treated at the lower doses of mercury. The immunological defects were consistent with altered T-cell function as evidenced by decreases in both T-cell mitogen, mixed leukocyte responses and T-cell mediated host resistance parameters. There was a particular association between the T-cell defects and inhibition of thymic pyruvate kinase, the rate limiting enzyme for glycolysis. The differences in the pattern of enzyme responses among lymphoid organs implied that two mechanisms of mercury toxicity were operative--one at high concentrations that caused physicochemical enzyme inhibition and another at low concentrations that caused indirect enzyme inhibition. *In vitro* studies have established that immunosuppression is due to binding of intracellular sulfhydryls since addition of cell-penetrating sulfhydryl-containing compounds but not non-penetrating are capable of protection. Further *in vitro* studies are planned for $HgCl_2$ to elucidate the potential mechanisms of mercury-induced immunosuppression.

Female mice treated with diethylstilbestrol, a non-steroidal estrogenic compound, were found to develop severe thymic atrophy, profound suppression of lymphocyte function and activation of resident peritoneal macrophages. A similar degree of immune dysfunction can be obtained by treating mice with 17-β-estradiol at approx. 10-fold greater doses. Administration of estrogenic mycotoxins (e.g. zearalenol) induces a similar degree of macrophage activation as DES, on a molar basis, without the concommitant suppression of lymphocyte responses. These functional disassociations are being further examined by evaluating response kinetics and inhibition of these responses by progesterone and anti-estrogenic compounds as well as evaluating other structurally similar compounds with A or D ring similarity. Furthermore, macrophage activation but not lymphocyte suppression can be reversed by adult thymectomy prior to chemical treatment. These studies indicated a direct relationship exists between estrogenic activity and suppression of lymphocyte activation which requires "A" region cell recognition in the inducing estrogenic compound. Thymic factors, however, are related to macrophage activation and which bone marrow suppression probably occurs through indirect mechanisms.

Ochratoxin-producing fungi are extremely widespread in nature having been frequently isolated from stored grain, oil seeds, damaged tobacco, insects and decayed vegatables. In preliminary studies, B6C3F$_1$ female mice were administered 4 dosages of ochratoxin (sc in corn oil) over a 2-week period at dosage levels of 5, 10 or 20 µg/g B.W. per dose. Marked myelotoxicity and suppression of humoral immunity are apparent at dosage levels which do not induce any signs of overt toxicity or altered liver enzymes. Further studies are underway to better characterize the immunotoxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A correlation has been clearly established between the administration of chemical immunosuppressants and an increased incidence of infectious diseases and neoplasia. The evidence for increased bacterial, viral, fungal and parasitic diseases in patients on chronic immunosuppressive chemicals has been well documented by Allen (Infection complicating neoplastic disease and cytotoxic therapy. In: Infection and the Compromised Host, 1976). Likewise, McKhann (Transplantation 8:209, 1971) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was 4.6-61 times higher than in the general population.

Studies in laboratory animals also have supported these clinical observations and demonstrated an enhanced incidence of UV-induced or benzopyrene-induced cancer in mice treated with immunosuppressive agents. The mechanistic relationship between altered host resistance and immune dysfunction is complex, poorly defined and of extreme importance. Chemicals of environmental concern have been recently shown to induce immunosuppression as evident by depressed antibody mediated immunity, cell-mediated immunity or MØ dysfunction in rodents following sublethal exposure. Some of the chemicals which induce immunologic effects in rodents include 2,3,7,8-tetrachlorodibenzo-p-dioxin, polychlorinated biphenyls, polybrominated biphenyls, gallic acid, DES, BP, hexachlorobenzene, pentachlorophenol, certain organo- and heavy metals. Some studies have indicated that exposure to certain chemicals can alter resistance to bacteria, viruses, parasites and transplantable tumor cells. Of major concern is the correlation of these immunologic findings with altered host susceptability and the extrapolation of these chemically induced immunobiologic effects to humans.

PUBLICATIONS

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Luster, M.I., Boorman, G.A., Dean, J.H., Lawson, L.D., Wilson, R.E., and Haseman, J.K.: Immunological alterations in mice following acute adult exposure to diethylstilbestrol. In Dean, J.H. and Padarathsingh, M.L. (Eds.): Biological Relevance of Immunosuppression. New York, Van Nostrand Reinhold, 1981, pp. 153-175, Chap. 11.

Dean, J.H., Luster, M.I., Boorman, G.A., Padarathsingh, M.L., Luebke, R.E., and Clements, M.E.: Host resistance models as endpoints in assessing immune alterations following chemical exposure: studies with diethylstilbestrol, cyclophosphamide and 2,3,7,8-tetrachlorodibenzo-p-dioxin. In Dean, J.H. and Padarathsingh,

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-09 STB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics of Chlorinated Xenobiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Hazel B. Matthews Research Chemist TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Toxicological Research and Testing Program

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary long-term goal of this work has been to correlate structure-activity relationships for halogenated hydrocarbons and determine how the degree and position of halogenation effects the absorption, disposition and bioaccumulation of these compounds. This work has established that whereas polar halogenated hydrocarbons may be excreted prior to metabolism, simple aryl halides are not excreted prior to metabolism to polar compounds and the rate of metabolism is limited by the availability of adjacent unsubstituted carbon atoms which are thought to facilitate metabolism via arene oxide intermediates. This work has also established that halogenated aromatics are readily absorbed from the gastrointestinal tract, that those compounds which are not polar or are not readily metabolized will persist in the tissues, that chronic exposure to persistent halogenated aromatics will result in bioaccumulation to toxic levels and that the ability to metabolize halogenated aromatics varies widely with species. Studies of halogenated biphenyl transport indicate that these and similar highly lipid soluble compounds are distributed throughout the body in association with hydrophobic sites on blood proteins.

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has utilized ^{14}C-labeled compounds to quantitate absorption, distribution, accumulation, metabolism and excretion of a series of nine polychlorinated biphenyls (PCBs), a polybrominated biphenyl (PBB) and two insecticides. PCBs have been studied in mice, rats and monkeys and the PBB and insecticides have been studied in rats. Studies of xenobiotic disposition have been conducted under conditions of normal feeding and starvation as well as acute versus multiple exposure. Analyses were facilitated by the use of a biological material's oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

MAJOR FINDINGS AND PROPOSED COURSE:

1) The findings described here and in previous years have provided the basis for the following conclusions for simple halogenated aromatics: a) their lipid solubility facilitates absorption, b) their half-lives are determined by their polarity or the rate at which they are metabolized to polar compounds, c) their metabolism is determined by the position rather than the degree of halogenation, d) the major sites of accumulation are the liver, skin and adipose tissue, and the relative importance of the liver and adipose tissues as storage sites is determined by the polarity of the compound in question. Experience and reputation gained in the course of this work has resulted in a number of invitations to prepare and present reviews and symposium articles (see Publications).

2) Halogenated biphenyl transport by components of rat blood was studied under both in vivo and in vitro conditions. Fractionation of plasma components by gel filtration, ultracentrifugation and chromatography on a column of fine glass beads indicate that halogenated biphenyls are associated with each major class of plasma proteins but are most concentrated in the lipoproteins. A significant portion of the total halogenated biphenyl in whole blood is also associated with the cellular component. Halogenated biphenyls are readily exchanged between plasma and the cellular component and between lipoproteins and other classes of plasma proteins. Partition of a series of halogenated biphenyls between lipoproteins and other plasma proteins indicated that the relative affinity of a biphenyl for each fraction was proportional to the lipid solubility of the biphenyl involved. The major portion of the halogenated biphenyls in blood are not thought to be bound to specific sites on blood proteins but rather they are believed to be associated with hydrophobic sites on plasma proteins or the cellular component of blood. The rapid transfer of these compounds to tissues is thought to be by partition to similar sites on cellular proteins.

PROPOSED COURSE: Studies done to date have firmly established those factors which determine the disposition of simple halogenated aromatics. Future studies will be designed to expand this base of knowledge by determining how other types of substitutions, in addition to halogens, effect the metabolism and disposition of organic compounds. In addition, the effort to elucidate the biochemical mechanism(s) by which halogenated hydrocarbons exert their action will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated xenobiotics are the most toxic and persistent of the chemicals contaminating our environment. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and numerous industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man and may lead to more specific modes of treatment including better ways to accelerate removal of such compounds from the body.

PUBLICATIONS

Matthews, H.B.: Disposition of persistent halogenated hydrocarbons in higher animals. In: Khan, M.A.Q. and Stanton, R. H. (eds). Toxicology of Halogenated Hydrocarbons: Health and Ecological Effects. Pergamon Press, Inc. New York, N.Y. pp. 289-297, 1981.

Matthews, H. B.: Chapter 3. Aryl Halides. In: Jakoby, W. B., Bend, J. R., and Caldwell, S. (eds.): Metabolic Basis of Detoxication. Academic Press, Inc. New York. pp. 51-68, 1982.

Matthews, H. B. and Birbaum, L. S.: Factors affecting the disposition and persistence of halogenated furans and dioxins. In. Symposium Proceedings: International Symposium on the Chlorinated Dioxins and Related Compounds. Arlington, VA, 1981. In Press.

Matthews, H. B.: Metabolism of PCBs in Mammals: Routes of Entry, Storage and Excretion. In. Symposium Proceedings: International Symposium on PCBs in the Great Lakes. East Lansing, Mich. 1982. In Press.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE - Chicago, Illinois 60616
(NIH-NO1-ES-1-5000)

TITLE: Chemical Induced Immunotoxicity

CONTRACTOR'S PROJECT DIRECTOR: James Fenter, Ph.D.

PROJECT OFFICER (NIEHS): M.I. Luster, Ph.D.
Immunotoxicology Group Leader, STB, TRTP

DATE CONTRACT INITIATED: February 1, 1981

CURRENT ANNUAL LEVEL: $285,076

PROJECT DESCRIPTION

OBJECTIVE: The objective of this contract encompasses efforts to develop improved assay methodology for measuring altered host resistance and immunological impairment in rodents exposed to chemicals of environmental concern, interlaboratory assay validation, and evaluation of selected chemicals to alter immune functions and host resistance to challenge with infectious agents or tumor cells. There are 3 major tasks involved in this project. They include: (i) evaluation of methods for evaluating host resistance to bacteria, viruses, animal parasites and transplantable tumors; (ii) establishment and proficient demonstration of a standardized set of immunologic tests and (iii) integration and validation of the test systems for altered host resistance and immunological function using at least 5 chemicals selected by NIEHS.

METHODS EMPLOYED:

I. Host Resistance Assays:

Altered susceptibility to challenge with various infectious agents are being examined in mice following exposure to chemicals of environmental concern including diethylstilbestrol, cadmium chloride and pentachlorophenol. A wide range of infectious agents are being employed for study and development as models, for which considerable information is available concerning the operative host resistance mechanisms. The original selected group of organisms include Listeria, Streptococcus and Klebsiella as the 3 bacteria, influenza and Herpes simplex as the two viruses, Trichinella spiralis as the parasite and the B16F10 as the transplantable tumor.

II. Immune Function Tests:

Evaluation of the following immune function assays are underway: (1) Lymphocyte proliferation to mitogens and allogenic leukocytes; (2) Antibody plaque forming cell response to a T cell-dependent antigen (both direct and indirect); (3) Quantitation of serum immunoglobulin levels; (4) Delayed hypersensitivity responses using radioisotopic assays; (5) Assays for macrophage function including RES clearance, tumor cell cytostasis, enzyme activity and phagocytosis.

III. Standard Toxicology:

Evaluation of body weight, lymphoid organ weight, selected histopathology, hematology profile and activities of selected liver enzymes are included to relate the toxic effects of chemical exposure on immune dysfunction.

MAJOR FINDINGS AND PROPOSED COURSE: The contractors have satisfactorily completed studies with DES and have made substantial progress in the development, validation and refinement of the immunologic and host resistance assays as outlined in the 1st year goal of this 4 year contract. Some of the more advanced models of host resistance including the Listeria monocytogenes, Herpes simplex virus, Trichinella spiralis challenge and B16-F10 tumor susceptability models have been shown to be highly sensitive indicators of immunotoxicity in that increased susceptibility occurs following challenge in chemically treated mice without any evidence of general toxicity. Most of the immune function test assays have been validated and found to be sensitive and reproducible techniques to detect chemical-induced immunotoxicity while others are still being modified. Several of the techniques that are being modified include serum immunoglobulin concentrations and delayed hypersensitivity response. It is recommended that the contractors move as rapidly as possible to complete the "mopping-up" part of the immune assays. More emphasis should be placed in year 2 of this contract in refining macrophage function assays and developing a host resistance panel. Studies utilizing $CdCl_2$ should be in progress and be completed by August 1982. The overall recommendation is that the contractor develop priorities for establishing those immune and host resistance assays that appear appropriate for the NTP panel.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A correlation has been clearly established between the administration of chemical immunosuppressants and an increased incidence of infectious diseases and neoplasia. The evidence for increased bacterial, viral, fungal and parasitic diseases in patients on chronic immunosuppressive chemicals has been well documented by Allen (Infection complicating neoplastic disease and cytotoxic therapy. In: Infection and the Compromised Host, 1976). Likewise, McKhann (Transplantation 8:209, 1971) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was 4.6-61 times higher than in the general population.

Studies in laboratory animals also have supported these clinical observations and demonstrated an enhanced incidence of UV-induced or benzopyrene-induced cancer in mice treated with immunosuppressive agents. The mechanistic relationship between altered host resistance and immune dysfunction is complex, poorly defined and of extreme importance. Chemicals of environmental concern have been recently shown to induce immunosuppression as evident by depressed antibody mediated immunity, cell-mediated immunity or MØ dysfunction in rodents following sublethal exposure. Some of the chemicals which induce immunologic effects in rodents include 2,3,7,8-tetrachlorodibenzo-p-dioxin, polychlorinated biphenyls, polybrominated biphenyls, gallic acid, DES, BP, hexachlorobenzene, pentachlorophenol, certain organo- and heavy metals. Some studies have indicated that exposure to certain chemicals can alter resistance to bacteria, viruses, parasites and transplantable tumor cells. Of major concern is the correlation of these immunologic findings with altered host susceptability and the extrapolation of these chemically induced immunobiologic effects to humans.

MEDICAL COLLEGE OF VIRGINIA - Richmond, Virginia 23298
(NIH-NO1-ES-1-5001)

TITLE: Chemical Induced Immunotoxicity

CONTRACTOR'S PROJECT DIRECTOR: Page S. Morahan, Ph.D.

PROJECT OFFICER (NIEHS): M.I. Luster, Ph.D.
Immunotoxicology Group Leader, STB, TRTP

DATE CONTRACT INITIATED: February 1, 1981

CURRENT ANNUAL LEVEL: $251,053

PROJECT DESCRIPTION

OBJECTIVE: The objective of this contract encompasses efforts to develop improved assay methodology for measuring altered host resistance and immunological impairment in rodents exposed to chemicals of environmental concern, interlaboratory assay validation and evaluation of selected chemicals to alter immune functions and host resistance to challenge with infectious agents or tumor cells. There are 3 major tasks involved in this project. They include: (i) evaluation of methods for evaluating host resistance to bacteria, viruses, animal parasites and transplantable tumors; (ii) establishment and proficient demonstration of a standardized set of immunologic tests and (iii) integration and validation of the test systems for altered host resistance and immunological function using at least 5 chemicals selected by NIEHS.

METHODS EMPLOYED:

I. Host Resistance Assays:

Altered susceptibility to challenge with various infectious agents are being examined in mice following exposure to chemicals of environmental concern including diethylstilbestrol, cadmium chloride and pentachlorophenol. A wide range of infectious agents are being employed for study and development as models, for which considerable information is available concerning the operative host resistance mechanisms. The original selected group of organisms include EMC and herpes simplex type 2 viruses; S. pneumoniae, E. coli, and L. monocytogenes as the bacteria; P. berghei as a parasite and the B16 melanoma as the transplantable tumor.

II. Immune Function Tests:

Evaluation of the following immune function assays are underway: (1) Lymphocyte proliferation to mitogens and allogenic leukocytes; (2) Antibody plaque forming cell response to a T cell-dependent antigen (both direct and indirect); (3) Quantitation of serum immunoglobulin levels; (4) Delayed hypersensitivity responses using radioisotopic assays; (5) Assays for macrophage function including RES clearance, tumor cell cytostasis, enzyme activity and phagocytosis.

III. Standard Toxicology:

Evaluation of body weight, lymphoid organ weight, selected histopathology, hematology profile and activities of selected liver enzymes are included to relate the toxic effects of chemical exposure on immune dysfunction.

MAJOR FINDINGS AND PROPOSED COURSE: The contractors have satisfactorily completed studies with DES and have made substantial progress in the development, validation and refinement of the immunologic and host resistance assays as outlined in the 1st year goal of this 4 year contract. Some of the more advanced models of host resistance including the L. monocytogenes, herpes simplex virus, cryptococcus neoformans, encephalomyocarditis virus and B16F10 tumor models have been shown to be highly sensitive indicators of immunotoxicity in that increased susceptibility occurs following challenge in chemically treated mice without any evidence of general toxicity. Most of the immune function test assays have been validated and found to be sensitive and reproducible techniques to detect chemical-induced immunotoxicity while others are still being modified. Several of the techniques that are being modified include serum immunoglobulin concentrations and delayed hypersensitivity response. It is recommended that the contractors move as rapidly as possible to complete the "mopping-up" part of the immune assays. More emphasis should be placed in year 2 of this contract in refining macrophage function assays and developing a host resistance panel. Studies utilizing $CdCl_2$ should be in progress and be completed by August 1982. The overall recommendation is that the contractor develop priorities for establishing those immune and host resistance assays that appear appropriate for the NTP panel.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A correlation has been clearly established between the administration of chemical immunosuppressants and an increased incidence of infectious diseases and neoplasia. The evidence for increased bacterial, viral, fungal and parasitic diseases in patients on chronic immunosuppressive chemicals has been well documented by Allen (Infection complicating neoplastic disease and cytotoxic therapy. In: Infection and the Compromised Host, 1976). Likewise, McKhann (Transplantation 8:209, 1971) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was 4.6-61 times higher than in the general population.

Studies in laboratory animals also have supported these clinical observations and demonstrated an enhanced incidence of UV-induced or benzopyrene-induced cancer in mice treated with immunosuppressive agents. The mechanistic relationship between altered host resistance and immune dysfunction is complex, poorly defined and of extreme importance. Chemicals of environmental concern have been recently shown to induce immunosuppression as evident by depressed antibody mediated immunity, cell-mediated immunity or MØ dysfunction in rodents following sublethal exposure. Some of the chemicals which induce immunologic effects in rodents include 2,3,7,8-tetrachlorodibenzo-p-dioxin, polychlorinated biphenyls, polybrominated biphenyls, gallic acid, DES, BP, hexachlorobenzene, pentachlorophenol, certain organo- and heavy metals. Some studies have indicated that exposure to certain chemicals can alter resistance to bacteria, viruses, parasites and transplantable tumor cells. Of major concern is the correlation of these immunologic findings with altered host susceptability and the extrapolation of these chemically induced immunobiologic effects to humans.

Research Triangle Institute
NO1-ES-1-5007

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: A. Robert Jeffcoat, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: July 15, 1981

CURRENT ANNUAL LEVEL: $238,059

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or intramural scientists at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism, and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest and metabolites. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) Studies of the absorption, metabolism and clearance of the sparingly soluble dye intermediate 1-amino-2,4-dibromoanthraquinone-^{14}C (ADQ) revealed that gastrointestinal absorption of this compound was highly dose dependent. Based on $^{14}CO_2$ exhaled following ADQ administration, gastrointestinal absorption decreased from approximately 90% at a dose of 2 mg/kg to 21% at 120 mg/kg to approximately 2% at 814 mg/kg. Therefore, the total ADQ absorbed at the two higher doses was not significantly different and these results have direct relevance to studies involving chronic administration of ADQ. That portion of the ADQ dose which was absorbed from the gastrointestinal tract was readily metabolized and excreted in bile and subsequently in feces. There was no evidence of ADQ accumulation in tissues.

2) A study of the metabolism and disposition demonstrated following intravenous administration of 0.76 mg/kg [^{14}C] cyclohexane to adult male Fischer 344 rats, 54% of the dose was excreted in the breath in the first hr, 80% in 24 hr, and 83% in 72 hr. After oral administration at doses of 2000, 1000, 200 and 100 mg/kg, 78, 76, 62 and 63%, respectively, of the dose was excreted in the breath over

72 hr with the maximum rate of excretion generally occurring from 2-8 hr. In the same experiments 12, 15, 29 and 29%, respectively, of the dose was excreted in urine, compared to 14% excreted in urine following the intravenous dose. Greater excretion of polar metabolites in urine following the smaller oral doses is attributed to relatively greater metabolism in liver following absorption from the gastrointestinal tract. No significant excretion of ^{14}C in feces was observed. Cyclohexane accounted for 93-99% of the radiolabel excreted in breath, but less than 0.1% of the radiolabel in the urine. A maximum of 0.04-0.4% of the dose was excreted in breath as the more toxic cyclohexanone or 0.09-0.6% as cyclohexanol. Less than 0.1% of the dose was excreted in the urine as either of these compounds. Cyclohexane was concentrated primarily in adipose tissues, but these concentrations were very transient and total excretion was quite rapid.

3) The disposition of a commonly used dye ,CI Vat Blue 1 (Blue 1), following oral, dermal and iv administration has been studied in the rat. The Blue 1 used contained a ^{14}C label and was as pure as could be obtained, approximately 85% pure. The results of this study indicate that Blue 1 is poorly absorbed following both oral and dermal absorption. This is due primarily to the extreme insolubility of this compound in biological media. The form administered also plays an important role in absorption. For example, dermal absorption of Blue 1 was approximately 1.7 $\mu g/cm^2$/week following dermal administration of 550 $\mu g/cm^2$ of dry dye, and 5.2 $\mu g/cm^2$/week when applied in a polyethylene glycol based ointment. Disposition studies following iv administration of a small dose, 0.22 mg/kg, indicated that this compound was slowly metabolized and eliminated primarily in feces. Blue 1 may have some potential for bioaccumulation with chronic exposure.

PROPOSED COURSE:

1. A study of the disposition and metabolism of 1,3-dichloro-5,5'-dimethylhydantoin will be done in the rat.

2. Additional compounds and classes of compounds will be studied as requested by personnel in the NIEHS intramural research program and/or the NTP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP, and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies on such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data to complement studies of toxic xenobiotics which will be done under the NTP or in the NIEHS intramural program.

Southern Research Institute
NO1-ES-1-5008

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: Donald L. Hill, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: July 15, 1981

CURRENT ANNUAL LEVEL: $218,672

PROJECT DESCRIPTION

OBJECTIVES: The object of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or intramural scientists at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism, and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest and metabolites. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with orgainic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) The disposition of Hexachlorocyclopentadiene ^{14}C (HCP) was studied in the rat following administration of oral or iv doses or inhalation of the vaporized chemical. It was determined that following oral administration most of the HCP reacted with stomach and gut contents and was never absorbed as such from the gastrointestinal tract. Following iv administration HCP reacted with blood components, primarily hemoglobin, to form inextractable complexes. Following inhalation HCP reacted with lung tissue in addition to being absorbed and distributed to most of the major tissues. The reactivity of HCP in biological systems was confirmed in vitro by demonstrating that it forms inextractable complexes when incubated with blood, liver or feces. The reactivity of HCP in the gastrointestinal tract which decreases its absorption versus that observed following inhalation explains why this compound is much more toxic when inhaled than when ingested. The results of this study are being prepared for publication.

2) The gastrointestinal (GI) absorption of a series of highly insoluble compounds is being studied in the rat. The first chemicals in the series are a group of three pigments, CI Pigment Red 3, CI Pigment Red 23 and CI Pigment Yellow 74. The

compounds studied are not radiolabeled and each study has required the development of a highly sensitive high-pressure liquid chromatographic assay. Studies to date have determined that GI absorption of intact pigments is minimal. Even after the administration of large doses, 60-600 mg/kg, tissue levels of these pigments are below the limits of detection, 5 ppb. The effect of microbial action on these chemicals and the possibility of absorption of the resulting microbial metabolites is also being investigated.

PROPOSED COURSE:

1. Studies on the effect of solubility to GI absorption of xenobiotics will be continued.

2. Additional compounds and classes of compounds will be studied as requested by personnel in the NIEHS Intramural Research Program and/or the NTP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP, and the NIEHS to detemine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies on such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data to complement studies of toxic xenobiotics which will be done under the NTP or in the NIEHS intramural program.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC.
(NIH-NO1-ES-1-5011)

TITLE: Sperm Morphology and Vaginal Cytology Evaluation

CONTRACTOR'S PROJECT DIRECTOR: P.S. Sabharwal, Ph.D.
President, EHRT

PROJECT OFFICER (NIEHS): James C. Lamb, IV, Ph.D.
Head, Fertility and Reproduction Group, STB, TRTP

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $97,588

PROJECT DESCRIPTION

OBJECTIVES: This contract was designed to supply a method for screening chemicals for reproductive toxicity. It standardizes and centralizes the collection of data collected from studies run in the numerous testing laboratories in the Bioassay Program. The system allows for the collection of reproductive toxicity data without purchasing additional animals, test chemical or animal care expenses. This arrangement also facilitates interstudy comparisons of reproductive toxicity.

METHODS EMPLOYED: Approximately twenty-five new chemicals per year begin testing in the Bioassay Program. The special reproductive toxicity testing screens used include sperm concentration, motility and morphology in male rats and mice and vaginal cyclicity in female rats and mice. The NTP bioassay testing laboratories collect the specimens, prepare the slides and ship them to this NTP-designated laboratory. EHRT is responsible for providing technical direction, evaluation, quality assurance, data summary and reports and slide inventory and storage.

MAJOR FINDINGS AND PROPOSED COUSE: Technical direction has been given by EHRT to bioassay laboratories before any slides have been collected. This should help assure uniformity in data collection and slide preparation. Slides will be sent to EHRT over the remainder of the contract; the protocol may be modified as the testing continues.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This reproductive toxicity testing system is an important component of the National Toxicology Program's Reproductive and Developmental Toxicology Program. This system represents an effort to effectively use animals which are already on test in the Bioassay Program and thereby eliminate redundant animal dosing, care and necropsy and enhance our ability to identify reproductive toxicants. EHRT is responsible for assisting in the coordination of this extensive effort and assuring high quality specimen collection and data analyses. These studies serve as a unique and cost-effective prescreening system for reproductive toxicology.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC.
(NIH-NO1-ES-2-5013)

TITLE: Fertility Assessment by Continuous Breeding

CONTRACTOR'S PROJECT DIRECTOR: P.S. Sabharwal, Ph.D.
President, EHRT

PROJECT OFFICER (NIEHS): James C. Lamb, IV, Ph.D.
Head, Fertility and Reproduction Group, STB, TRTP

DATE CONTRACT INITIATED: January 29, 1982

CURRENT ANNUAL LEVEL: $244,115

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to evaluate a new reproductive toxicology testing system.

METHODS EMPLOYED: This reproductive toxicology testing system employs an extended chemical exposure and a protocol which includes the mating of continuously-exposed male and female mice. Mating pairs will be housed together for 100 days and offspring will be counted to determine an index of cumulative fertility. The system allows for testing offspring collected between 100 and 120 days, if the parental generation has not been adversely affected by the chemical exposure. The test system may also be used to identify the affected sex or study various target organ response with a special necropsy which focuses on reproductive target organs. The special organ response studies may include sperm concentration, sperm morphology, vaginal cytology and plasma hormone analyses.

MAJOR FINDINGS AND PROPOSED COURSE: This contract was awarded in the second quarter of FY 1982. Chemical testing has begun and will continue on eight chemicals until FY 1983. This phase of testing is designed to evaluate the testing system's utility.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is an essential element in the National Toxicology Program's initiative in reproductive toxicology test development and validation. This is a new testing protocol which will be compared to other, more expensive testing systems and should lead us to new and improved reproductive toxicity testing systems.

RESEARCH TRIANGLE INSTITUTE
(NIH-NO1-ES-2-5014)

TITLE: Fertility Assessment by Continuous Breeding

CONTRACTOR'S PROJECT DIRECTOR: Jerry R. Reel, Ph.D.
Director, Toxicology and Life Sciences Division

PROJECT OFFICER (NIEHS): James C. Lamb, IV, Ph.D.
Head, Fertility and Reproduction Group, STB, TRTP

DATE CONTRACT INITIATED: January 27, 1982

CURRENT ANNUAL LEVEL: $253,943

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to evaluate a new reproductive toxicology testing system.

METHODS EMPLOYED: This reproductive toxicology testing system employs an extended chemical exposure and a protocol which includes the mating of continuously-exposed male and female mice. Mating pairs will be housed together for 100 days and offspring will be counted to determine an index of cumulative fertility. The system allows for testing offspring collected between 100 and 120 days, if the parental generation has not been adversely affected by the chemical exposure. The test system may also be used to identify the affected sex or study various target organ response with a special necropsy which focuses on reproductive target organs. The special organ response studies may include sperm concentration, sperm morphology, vaginal cytology and plasma hormone analyses.

MAJOR FINDINGS AND PROPOSED COURSE: This contract was awarded in the second quarter of FY 1982. Chemical testing has begun and will continue on eight chemicals until FY 1983. This phase of testing is designed to evaluate the testing system's utility.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is an essential element in the National Toxicology Program's initiative in reproductive toxicology test development and validation. This is a new testing protocol which will be compared to other, more expensive testing systems and should lead us to new and improved reproductive toxicity testing systems.

University of Oregon Health Sciences Center
School of Medicine
Department of Pharmacology
NO1-ES-7-2126

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: Robert K. Lynn, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, TRTP

DATE CONTRACT INITIATED: September 30, 1977

CURRENT ANNUAL LEVEL: $157,790

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or scientists in the intramural program at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner, and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS:

1) The metabolism, distribution and excretion of 3,3'-dimethoxybenzidine-^{14}C have been studied in the rat. This compound was readily absorbed from the gastrointestinal tract, metabolized and excreted primarily in bile and subsequently in feces. 3,3'-Dimethoxybenzidine was metabolized to more than 20 metabolites most of which are excreted in the form of glucuronide and sulphate conjugates. N-acetyl metabolites of 3,3'-dimethoxybenzidine are formed, but they are not as prevalent as was previously observed for benzidine. This work is complete and a manuscript describing it has been completed and submitted for publication.

2) A disposition study of 3,3'-dimethylbenzidine-^{14}C has been completed in the rat. This benzidine congener is also readily absorbed and rapidly metabolized. 3,3'-Dimethylbenzidine is excreted almost exclusively in the form of metabolites. Excretion in bile and subsequently in feces is approximately three times as great

as excretion in urine. The metabolites excreted include glucuronide, sulphate and N-acetyl conjugates. A manuscript describing the work has been prepared and submitted for publication.

3) Direct Blue 6, a benzidine based dye, previously synthesized by this contractor to contain a ^{14}C label in the benzidine nucleus has been the subject of a metabolism and disposition study in the rat. The results of this study indicate that the intact dye is not well absorbed from the gastrointestinal tract. However, the azo linkages binding the ^{14}C benzidine nucleus to the chromophores is subject to reduction by intestinal microbes resulting free benzidine. Benzidine is absorbed from the gastrointestinal tract and metabolized as described previously. That portion of the Direct Blue 6 dose which is not metabolized by intestinal microbes is excreted in feces.

4) ^{14}Acid Red 114, a 3,3'-dimethylbenzidine based dye, has been synthesized with a ^{14}C label in the 3,3'-dimethylbenzidine nucleus. The radiochemical purity of this radiolabeled dye is greater than 97% as determined by HPLC.

5) A study of the chemical disposition of radiolabeled Acid Red 114 has been completed in the rat. This dye is subject to metabolism by azo reduction to release the ^{14}C labeled 3,3'-dimethylbenzidine which is metabolized and excreted as described above. The relatively large dye molecule is not absorbed intact and most of an oral dose which does not undergo azo reduction by intestinal microbes is excreted intact in the feces.

6) The metabolism and disposition of 3,3'-dichlorobenzidine ^{14}C has been studied in the rat. This compound is readily absorbed, and extensively metabolized. Greater than 70% of the dose is excreted in bile and subsequently in the feces in the form of mono- and diacetyl-3,3'-dichlorobenzidine, N-glucuronides and hydroxylated metabolites. The parent compound has been determined to be mutagenic in the Ames system in the presence of S-9 and some of the metabolites of this compound appear to be even more mutagenic in this system than the parent compound. The results of this study are being prepared for publication.

PROPOSED COURSE: This contract will expire at the end of the current fiscal year. Prior to that time each of the major findings described will be completed, reports will be submitted, and the results will be prepared for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies of such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data which will complement studies of toxic xenobiotics under the NTP or in the NIEHS intramural program.

PUBLICATIONS

Lynn, R. K., Wong, K., Garvie-Gould, C. and Kennish, J. M.: Disposition of the flame retardant, tris 1,3-dichloro-2-propyl)-phosphate, in the rat. Drug Metab. Dispos. 9: 434-441, 1981.

Lynn, R. K., Garvie-Gould, C., Wong, K., and Kennish, J. M.: Metabolism distribution and excretion of the flame retardant, tris(2,3-dibromopropyl) phosphate(Tris-BP) in the rat: Identification of mutagenic and nephrotoxic metabolites. Toxicol. Appl. Pharmacol. 63: 105-119, 1982.

Elliott, W. C., Lynn, R. K., Houghton, D. C., Kennish, J. M., and Bennett, W. M. Nephrotoxicity of the flame retardant, tris(2,3-dibromopropyl)phosphate, and its metabolites. Toxicol. Appl. Pharmacol. 62: 179-182, 1982.

Lynn, R. K., Gould, C. G., Scott, K. F., Milan, D. F. and Rodgers, R. M.: Disposition of the human carcinogen benzidine in the rat: Characterization of mutagenic urinary and biliary metabolites. Toxicol. Appl. Pharmacol. In Press.

Arizona Board of Regents
University of Arizona
Tucson, Arizona 85724
NO1-ES-8-2130

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: I. Glenn Sipes, PH.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, TRTP

DATE CONTRACT INITIATED: September 15, 1978

CURRENT ANNUAL LEVEL: $158,798.00

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or intramural scientists at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest and metabolites. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) The metabolism and disposition of o-nitroanisole ^{14}C (ONA) was studied in the rat following oral, iv and dermal exposure. ONA was absorbed following either oral or dermal exposure. The half-life for dermal absorption was approximately 20 hr. Following iv administration ONA was rapidly distributed to all tissues assayed, readily metabolized and eliminated in a biphasic manner with an initial half-life of 1 to 2 hr and a terminal half-life of approximately 4 days. The initial component of the biphasic elimination of ONA accounted for approximately 90% of the dose, and there was little evidence of ONA accumulation or persistence in tissues. Following absorption, ONA distribution and metabolism was independent of route of administration or dose in the range of 5-50 mg/kg. However, at an oral dose of 500 mg/kg there was some evidence that the mechanisms responsible for ONA metabolism and/or elimination were saturated. ONA was excreted primarily in urine, 85%, almost exclusively, 99%, as a variety of metabolites. The major metabolites were o-nitrophenylsulfate, 63%, and o-nitrophenylglucuronide, 11%. These results are being prepared for publication.

2) p-Chloroaniline - ^{14}C (PCA) was the subject of metabolism and disposition studies in the rat. This chemical was readily absorbed from the gastrointestinal tract and very rapidly metabolized. Clearance from tissues was also quite rapid and virtually all of the PCA excreted was in the form of metabolites. The elimination half-life for PCA derived radioactivity from most tissues was 1.5 to 3 hr and the major route of elimination was in urine (>80%). The major metabolite of PCA was p-chloroacetanilide. Disposition kinetics for PCA appear to be independent of route of administration and dose in the range studied (0.3 to 30 mg/kg).

3) The metabolism and disposition of 1,2,3-trichloropropane-^{14}C (TCP) was studied in the rat following oral doses of 0.32, 3.2 and 32 mg/kg and an iv dose of 3.6 mg/kg. No effect of dose on metabolism and disposition of TCP was observed in the range studied. Following iv administration TCP was cleared from blood in a biphasic manner with half-lives of 0.25 and 23 hours. The component of the decay curve having the shorter half-life accounted for most of the dose. The highest tissue concentrations were observed in adipose tissue, but this and all other tissue concentrations were transient and 78% of the dose was excreted within 8 hr following iv administration. With exception of 5% of the dose eliminated in the exhaled air as the parent compound, TCP was cleared form the body as metabolites. The major route of excretion was urine which accounted for the elimination of 46% of the dose primarily as glutathione conjugates, 25% of the dose was metabolized to CO_2 and exhaled and 23% was eliminated in feces.

4) p-Nitrotoluene-^{14}C (PNT) was readily absorbed from the gastrointestinal tract of rats, rapidly metabolized and excreted primarily in urine. No effect of dose on absorption, metabolism or excretion was observed at the doses studied, 1,10 and 100 mg/kg. Following iv administration PNT was metabolized very rapidly and no parent compound could be detected in any tissue 4 hr post administration. PNT metabolites were rapidly cleared from the body and 70% of the dose was excreted in urine within 4 hr after administration. Excretion in feces was minimal, but studies with bile cannulated animals indicate that a significant portion of the dose is eliminated in bile prior to enterohepatic circulation and subsequent elimination in urine. This observation may be relevant in view of the fact that enterohepatic circulation is thought to contribute to the carcinogenicity of dinitrotoluene. The major metabolites of PNT have been identified as 4-nitrobenzoic acid and 2-hydroxy-4-nitrotoluene. These metabolites account for 50-60% of the PNT derived radioactivity excreted in urine. Minor metabolites are primarily conjugates and include the glucuronide of p-nitrobenzyl alcohol.

5) Work on studies of gallium arsenide (GA) disposition has concentrated on methods for the preparation of GA for administration and analysis of these elements in biological media. It has been determined that the proper oxidation state can be maintained if GA is ground under nitrogen to a powder of 1-5 micron particle size. Most forms of GA are insoluble, but in vitro studies of GA in the powdered form indicate that it has rapid and substantial solubility in buffered mediums. A technique has been devised for the analysis of arsenic in tissues in ng quantities. The method converts arsenic to arsine gas for analysis by atomic absorption and has a sensitivity of 2-5 parts per billion. Atomic absorption is also used for analysis of galium, but present limits of the assay are only 1-2 parts per million. Work to improve this assay and study the disposition of GA in rats is continuing.

6) A continuing project to study the metabolism of three polychlorinated biphenyls (PCBs) in in vitro preparations from dog, monkey and human liver for comparison with in vivo data is yielding data which corroborates data obtained in previous in vivo studies. That is, that the species as well as the degree and position of chlorination have profound effects on the rate at which PCBs are metabolized. Enzyme kinetic studies of the metabolism of 4,4'-dichlorobiphenyl (4,4) and 2,3,6,2',3',6'-hexachlorobiphenyl (236) by microsomal preparations from human liver yield the following values for 4,4 and 2,3,6 respectively:

Km = 10 μ molar
Vmax = 5.4 p mol/mg protein/min

Km = 0.4 μ molar
Vmax= 1.2 p mol/mg protein/min

The major metabolite formed from 4,4 was 3-hydroxy-4,4-dichlorobiphenyl. The major metabolites formed from 2,3,7 were 4 and 5-hydroxy-2,3,6,2',3',6'-hexachlorobiphenyl. Similar metabolites and kinetic parameters were obtained with microsomal preparations from monkey liver, but neither species was able to metabolize the third PCB 2,4,5,2',4',5'-hexachlorobiphenyl (2,4,5). On the other hand, microsomal preparations from dog liver can metabolize 4,4 and 2,3,7 more rapidly than those from human or monkey liver, and dog liver microsomes can metabolize 2,4,5 at an appreciable rate. The results of these studies indicate that the monkey is a better animal model for human metabolism of PCBs than the dog. Efforts to accurately predict rates of in vivo metabolism based on results obtained in vitro are continuing.

PROPOSED COURSE:

1. A comparative study of the metabolism and disposition of triortho-, trimeta- and triparacresylphosphate will be done in the rat and at least one species which is sensitive to the neurotoxicity of triorthocresyl phosphate.

2. A structure-activity study will be conducted for the dermal absorption of phthalic acid, dimethyl-, diethyl-, di-n-butyl-, diisobutyl-, di-n-hexyl-, di-n-octyl-, di(2-ethylhexyl)-, diisodecyl- and butyl benzyl phthalates plus 3 or 4 monoalkyl phthalates.

3. A study of the disposition of butyl benzyl phthalate will be done in the rat.

4. A comparative metabolism and disposition study, rat versus mouse, will be done for diallyl phthalate.

5. The work on in vitro versus in vitro versus in vitro metabolism and extrapolation will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP, and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies on such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and

kinetic data to complement studies of toxic xenobiotics which will be done under the NTP or in the NIEHS intramural program.

PUBLICATIONS

Miller, M. J., Carter, D. E. and Sipes, I. G.: Pharmacokinetics of arylamide in Fischer 344 rats. Toxicol. Appl. Pharmacol. 63, 36-44, 1982.

Sipes, I. G., Slocumb, M. L., Chen, H-S. G. and Carter, D. E.: 2,3,6,2',3',6'-Hexachlorobiphenyl: Distribution, metabolism and excretion in the dog and monkey. Toxicol. Appl. Pharmacol. 62, 317-324, 1982.

Ryerson, B. A., Carter, D. E. and Sipes, I. G.: Comparison of ^{14}C-2,4,5,2',4',5'-hexachlorobiphenyl levels in different adipose tissues of dogs and monkeys. Fund. Appl. Toxicol. In Press.

Sipes, I. G., Slocumb, M. L., Perry, D. F. and Carter, D. E.: 2,4,5, 2',4',5', Hexachlorobiphenyl: Distribution, metabolism, and excretion in the dog and the monkey. Toxicol. Appl. Pharmacol. In Press.

MOUNT SINAI SCHOOL OF MEDICINE - New York, NY 10029
(NIH-NO1-ES-9-0004)

TITLE: Investigation of the Immunobiological and Toxicological Effects of PBB in Michigan Farmers and Chemical Workers

CONTRACTOR'S PROJECT DIRECTOR: J.G. Bekesi, Ph.D. and I. Selikoff, M.D.

PROJECT OFFICER (NIEHS): John A. Moore, D.V.M.

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL: $500,000

PROJECT DESCRIPTION

OBJECTIVES: Altered immune function has been reported following exposure to PBB in humans and rodents. Altered immune function is but one of several symptoms and conditions that have been reported in PBB exposed persons. Thus far, investigation of immunological dysfunction and symptoms, in conjunction with various measures of PBB exposure have not shown a clearly defined dose/response relationship. This may be because: 1) no such relationship exists; 2) current methods of estimating exposure based on body burden are inadequate for this purpose; 3) such a relationship will only emerge after considerable time has passed; or 4) only persons susceptible for other reasons show immunotoxicity.

The specific objectives of this study are as follows: 1) to verify the existence of previously reported immune dysfunction in an expanded population of Michigan farmers and chemical workers; 2) to relate any disturbance of immunity to detailed measures of PBB exposure, either historical or biochemical; 3) to investigate whether other reported symptoms, signs or conditions occur more frequently in those persons with immune dysfunction than those without; and 4) to characterize and define the underlying mechanism of the immune dysfunction observed to date.

METHODS EMPLOYED: Starting in the spring of 1980 a portion of the original 250-300 farm personnel examined in the 1976 Mt. Sinai survey and a similar group constitute a Michigan population group exposed to PBB through ingestion of food products were studied. Approximately 50-75 Wisconsin residents were evaluated for control (not exposed to PBB) purposes. In addition, a population of up to 90 Michigan chemical workers were evaluated to compare individuals who were directly exposed to PBB and those who realized their PBB exposure principally through ingestion of food products.

All individuals enrolled in this study received a standardized health evaluation with specific focus on parameters allegedly associated with PBB exposure. Specific focus parameters will comprise the following: immunologic evaluation; liver function, to include standard clinical chemistry enzymes; neurological and/or neuropsychiatric evaluation, to include specific attention to the relatively unique secondary hypersomnia that has been reported; qualitative and quantitative evaluation of porphyrins in urine; dermatologic examination; and to establish serum and fat organohalide body burdens (PBB, PCB, DDT, DDE, etc.) to correlate health status, including immune alterations with any or all chemicals

that constitute the organohalogen body burden. Also, the contractor will conduct experiments on the qualitative and quantitative compartmentalization of PBB in specific subsets of lymphocytes and attempt to correlate these with immune alterations. Finally, the contractor will characterize the original population to validate the previous data. During this final year, the contractor will extensively evaluate people demonstrating altered lymphocyte immune function with specific focus on a variety of factors such as, responsiveness of null cells to BRM's, extensive characterization of various T-lymphocyte subsets or surface markers and function, characterization of macrophage function, and investigation of the role of antibody synthesis and regulation.

MAJOR FINDINGS AND PROPOSED COURSE: During the previous years, immunological profiles and clinical evaluations were performed on 336 PBB-exposed Michigan dairy farmers and 29 Michigan chemical workers, along with a low level exposure group from the general population (117) and two negative control groups, one from the Michigan general population (48) and the other Wisconsin dairy farmers (41). The immunological data were subjected twice to peer review for technical soundness and possible clinical significance by an outside committee of clinical immunologists, chemists, and statisticians. The peer reviewers were satisfied with the technical quality of the data as well as the specimen collection, transport and double blind coding. The most significant *in vivo* observation was an enhanced delayed cutaneous hypersensitivity response to recall antigens (streptococcal and mumps antigens) in up to 50% of the exposed group. *In vitro* analysis of serum and lymphocytes revealed elevated immunoglobulin levels (IgG, IgM and IgA) in up to 30% of the exposed population, depressed lymphoproliferative responses to T-cell mitogens and allogeneic leukocytes, decreased number of thymus-dependent lymphocyte with an accompanying increase in cells lacking neither T- or B-cell markers (so called "null cells") in approximately 30% of PBB exposed individuals.

One problem, as noted by the review committee, in interpreting these findings is the failure to statistically segregate and correlate the various clinical findings to develop an abnormality profile in conjunction with PBB body burden. This aspect has been recommended to the contractor for completion during the final contract year.

A few individuals possessing the greatest immune dysfunction are being evaluated in greater detail at the Mt. Sinai Medical Center to better characterize these changes and examine the potential use of biological response modifiers to correct these alterations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The complex relationship between PBB exposure, current PBB body burden and altered immune function in man remains yet undefined. The health implications of the altered skit test reactivity, elevated immunoglobulin levels, altered lymphocyte surface marker expression and the observed T-lymphocyte dysfunction may have important long-term health consequences on immune surveillance against infectious agents, neoplastically transformed cells and autoimmune diseases.

PUBLICATIONS

Bekesi, J.G., Holland, J.S., Anderson, H.F., Fischbein, A.S., Rom, W., Selikoff, I.J.: Lymphocyte Function of Michigan Farmers Exposed to Polybrominated Biphenyls. Science 199: 1207, 1978.

Anderson, H.A., Wolff, M.S., Lilis, R., Holstein, E.C., Valciukas, J.A., Anderson, K.E., Petrocci, M., Sarkozi, L., and Selikoff, I.J.: Symptoms and Clinical Abnormalities Following Ingestion of Polybrominated-biphenyl-contaminated Food Products. Ann. New York Acad. Sci. 320: 684-702, 1979.

Bekesi, J.G., Anderson, H.F., Roboz, J.P., Roboz, J., Fischbein, A.S., Selikoff, I.J., Holland, J.S.: Immunological Dysfunction Among PBB Exposed Michigan Dairy Farmers. Ann. New York Acad. Sci. 320: 717, 1979.

Bekesi, J.G., Roboz, J., Anderson, H.F., Roboz, J.P., Fischbein, A.S., Selikoff, I.J., Holland, J.S.: Impaired Immune Functions and Identification of Polybrominated Biphenyls (PBB) in Blood Compartments of Exposed Michigan Dairy Farmers and Chemical Workers. Drug and Chem. Toxicol. 2: 179, 1979.

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Roboz, J., Suzuki, R., Holland, J.S., Anderson, H.F., Selikoff, I.J., Bekesi, J.G.: Mass Spectrometry Identification and Quantification of Polybrominated Biphenyls in Blood Compartments of Exposed Michigan Chemical Workers. Environ. Path. and Toxicol. 3: 363, 1980.

Bekesi, J.G., Anderson, H., Roboz, J.P., and Selikoff, I.J.: Investigation of the Immunobiological Effects of PBB in Michigan Farmers. In Dean, J.H. and Padarathsingh, M.L. (eds.): Biological Relevance of Immunocompetence. New York, Van Norstrand Reinhold, 1981.

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Roboz, J., Greaves, J., Holland, J.F., and Bekesi, J.G.: Determination of Polybrominated Biphenyls in Serum by Negative Chemical Ionization Mass Spectrometry. Anal. Chem. 54: 1982.

Greaves, J., Bekesi, J.G., Roboz, J.: Halogen Anion Formation by Polybrominated Compounds in Negative Chemical Ionization Mass Spectrometry. Biomed. Mass Spec. (in press).

NORTHROP SERVICES, INC. - Research Triangle Park, NC 27709
NIEHS NO1-ES-79-0009

TITLE: Animal Research on the Inhalation Toxicology of Environmental Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Bernard Adkins, Ph.D.

PROJECT OFFICER (NIEHS): E.W. Van Stee, D.V.M., Ph.D.

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL (5 years): $2,663,653.00

PROJECT DESCRIPTION

OBJECTIVES: Conduct research in the inhalation toxicology of environmental chemicals using dynamic flow-through inhalation chambers designed for use with small laboratory animals. Exposures are conducted intermittently since the inhalation facility is not equipped for 24-hour inhalation exposures. Generate, monitor, characterize and control the generation of solid aerosols of asbestos and related natural and man-made fibers in 1-4 inhalation chambers as specified to support the research program of the National Toxicology Program. Design and place into operation a computer-assisted augmentation of the existing gas inhalation facility based on concepts and specifications provided by the Government. Conduct 2-year bioassay of naphthalene. Conduct a 2-year oncogenesis study in which groups of rats and hamsters receive NO_2 by inhalation and 2,6-dimethylmorpholine (DMM) in the drinking water, air plus DMM, NO_2 and plain drinking water, or air plus plain drinking water.

MAJOR FINDINGS AND PROPOSED COURSE: Site preparation by the Government is in progress and the development of the computer system is continuing as fast as the renovation proceeds. Completion of the Government renovation is expected to extend well into 1982. Computer-assisted operation of the facility should be well-along by 1983. Detailed protocols for the NO_2-DMM and Strain A/J mouse studies have been written and reviewed. The NO_2-DMM exposures will be completed during 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The implementation of computer-assisted inhalation facility operation represents an attempt to bring inhalation technology closer to present-day laboratory computer technology. Computer-assisted operation greatly enhances the accuracy and flexibility of the operation of the inhalation facility as well as the documentation of that operation, and greatly reduces manpower requirements, thus reducing the labor costs of facility operation. The potential in vivo interaction of NO_2 with heterocyclic amines represents a novel concept in environmental carcinogenesis. That detectable quantities of potentially carcinogenic nitrosamines can be formed in the bodies of laboratory animals that are given morpholine by gavage and exposed to NO_2 by inhalation has been reported. The biological significance of the phenomenon has been suggested by preliminary studies conducted at NIEHS. The results of these studies suggest the existence of a specific chemical link between exposure to NO_2 and tumor formation in mice (and possibly rats). Further, these studies pave the way for efforts to find

ways to interfere with elements of the in vivo process of oncogenesis with the long-term goal of identifying possible protective measures that might be taken to reduce the effects of this potential environmental hazard.

DEPARTMENT OF ENERGY - BROOKHAVEN NATIONAL LABORATORY
(NIEHS Interagency Agreement ES-9-0043)

TITLE: Evaluation of Repository Mechanics and Other Endpoints as Indices of Chemical Toxicity.

CONTRACTOR'S PROJECT DIRECTOR: R.T. Drew, Ph.D. and R.S. Kutzman, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director
National Toxicology Program

DATE INTERAGENCY INITIATED: July 16, 1979

CURRENT ANNUAL LEVEL: $350,000

PROJECT DESCRIPTION

OBJECTIVES: The interagency agreement is for the conduct of a research program for evaluation of respiratory mechanics and other endpoints as indices of chemical toxicity. Brookhaven National Laboratory will conduct investigations on six chemicals, one animal species, and three dose levels that: (a) compare changes in functional indices to changes determined through microscopic morphology; (b) assess the in vitro mutagenic potential of these chemicals using cytogenetic techniques including sister chromatid exchange; (c) determine lung connective tissue changes such as collagen and elastin; and (d) evaluate other selective toxicity parameters such as reproductive capacity, hematopoietic change, and organ function through use of serum chemistry.

METHODS EMPLOYED: Established techniques for evaluation of respiratory function tests are being used. The cytogenetic techniques including sister chromatid exchange are being used for assessment of in vivo mutagenic potential for the chemicals. Stepwise discriminant analysis is being used to select and linearly combine those pulmonary function and biochemical variables which best distinguish the four different exposure groups for each compound being studied.

MAJOR FINDINGS AND PROPOSED COURSE: Sixty-two exposures to either 0.2, 0.8, or 2.0 ppm ozone results in changes in pulmonary function at all exposure concentrations. Dynamic measurements are more sensitive indicators of ozone damage than static measurements. The maximum expiratory flow volume maneuver demonstrated air flow obstruction at low lung volume in all exposure groups. Multibreath nitrogen washout indicated abnormalities in lung ventilation-distribution at all exposure levels in contrast to the functional measurement, histological changes were only evident at the 2.0 ppm concentration of ozone. Biochemical changes were consistent with the above observations.

Similar studies were conducted with 0.4, 1.4, and 4.0 ppm acrolein. In contrast to ozone, acrolein did not affect the functional measurement in a dose-related fashion. At 4.0 ppm, functional measurement indicated an obstructive lesion of findings which were confirmed by bronchiolar epithelial necrosis and focal edema observed under the microscope. There was a greater variability in the response of rats exposed to acrolein than to ozone. At lower concentrations of acrolein, the flow volume maneuvers demonstrated flow higher than controls, possibly resulting from more rigid airways.

With chlorine, subchronic exposures to 0.5, 1.5, or 5.0 ppm did not result in marked pathologic lesions or functional changes. In spite of marked weight loss in the high dose group, the only functional change noted was a loss of elastic recoil indicative of a mild obstructive lesion.

Similar studies are currently underway with silica at 2, 10, and 20 mg/m^3. Pulmonary evaluations were conducted after three months and will be conducted after six months and again six months after a six month exposure. After three months of exposure, few functional changes were noted. The histological and biochemical data have not yet been evaluated. Studies are planned to expose rats to aerosols of cadmium chloride and also with tungsten carbide-cobalt mixtures. These studies will continue through FY 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the past decade, significant advances have been made in assessing pulmonary function of small animals. These studies present a systematic comparison between biochemical, functional, and anatomical changes resulting from exposure to a series of airborne pollutants. Completion of these studies will allow selection of appropriate end-points which indicate toxicity of a variety of environmental agents including gases and particles.

BIOMETRY AND RISK ASSESSMENT PROGRAM

BIOMETRY AND RISK ASSESSMENT PROGRAM
Summary Statement

The Biometry and Risk Assessment Program (BRAP) plans and conducts basic and applied environmental health oriented research in the areas of risk assessment, statistics biomathematics, and epidemiology. In addition it collaborates with scientists involved in the Toxicology Research and Testing Program, assuming responsibility for data management and statistical analysis. It also provides statistical, mathematical, data processing, and computer engineering support to other programs of the Institute; assists the Office of the Director in addressing specific health issues that bear on the welfare of the general public; and maintains an active association with peer groups in other federal agencies, academic institutions and private organizations with similar research interests.

The Biometry and Risk Assessment Program is organized into a Statistics and Biomathematics Branch (SBB), an Epidemiology Branch (EB), and a Computer Technology Branch (CTB). The Statistics and Biomathematics Branch conducts a broad research effort ranging from statistical analysis to biomathematical modeling aimed at developing new or improved methods for quantitative risk estimation, particularly in the areas of carcinogenesis, mutagenesis, and reproduction. Branch scientists maintain an active research program in statistical methodology relevant to design and analysis issues arising in laboratory experimentation, with special emphasis on toxicological screening assays. They also provide a comprehensive consulting service for the epidemiological component of the Biometry and Risk Assessment Program, and the National Toxicology Program and Intramural Research Program. The Epidemiology Branch initiates field studies of human disease, particularly chronic diseases, attributable to environmental pollutants; investigates the effects of environmental toxins on fetal and/or child development; and conducts basic and applied research in laboratory support methodology involved in the monitoring of human populations. The Computer Technology Branch operates the Institute's computer systems and the network of terminals connected to the various computers at NIH/DCRT; provides programming consultation services including software systems development to Institute personnel; maintains an active computer engineering group, which furnishes computing support to laboratory research activities in various branches; provides systems analysis and project management support to both Institute and NTP system development projects, and coordinates the Institute's word processing function.

COMPUTER TECHNOLOGY BRANCH

COMPUTER TECHNOLOGY BRANCH
Summary Statement

The Computer Technology Branch has the responsibility for providing computing and data processing support to NIEHS and the National Toxicology Program. This service may be thought of as consisting of three cooperating and interdependent efforts, namely computer operations and support programming, information systems development and computer engineering.

The computer operations and programming effort assumes the responsibility for maintaining NIEHS' PDP 11/70 and VAX 11/780 computer systems and a network of terminals connected to the various computers at NIH/DCRT, assisting the NIEHS community in its use of available computer systems, offering programming consultation services as required, furnishing software systems development capabilities to support intramural research efforts, and providing support and collaborative assistance to the computer engineering effort.

The information systems development effort consists of several projects that are concerned with the development of large, automated systems for both the Institute and the TRTP/NTP. Institute projects include efforts on behalf of the Office of Administrative Management and the Extramural Program. For the TRTP, projects underway include the Environmental Mutagenesis Information System, the Carcinogenesis Bioassay Data System, the Toxicology Data Management System (in cooperation with the National Center for Toxicological Research), and the Chemical Information and Tracking System. Management and coordination of the Institute's word processing function has recently been included among the responsibilities of this group.

Provision of computer engineering support to the laboratories of the Institute is also ongoing within the Computer Technology Branch. Solutions are being sought to engineering problems related to all aspects of computer hardware and software. Tasks within this effort have included the specification of minicomputers, peripherals, and vendor-supplied software; the design of timing devices and interfaces between minicomputers and laboratory instruments; and the development of software for control of experiments, data acquisition, data analysis, and data transfer.

LAWRENCE BERKELEY LABORATORY/UNIVERSITY OF CALIFORNIA
Berkeley, California 94720
(222Y01-ES-10066)

TITLE: Quantitative Species Extrapolation in Carcinogenesis

CONTRACTORS PROJECT DIRECTOR: Bruce N. Ames, Ph.D.

PROJECT OFFICER (NIEHS): David G. Hoel, Ph.D.
Program Director, BRAP

COLLABORATING INSTITUTE: Department of Energy

DATA CONTRACT INITIATED: April 1, 1981

CURRENT ANNUAL LEVEL: $208,500

PROJECT DESCRIPTION

OBJECTIVES: The objective of this research effort is to construct a large-scale, computerized data base employing information abstracted from existing research reports of animal cancer bioassays and to use this data base to investigate a variety of issues that are relevant to the general problem of quantitative species extrapolation in carcinogenesis. These issues include: (1) the similarity or lack of similarity of potency indices calculated from independent tests on the same compound, (2) the comparability of results for males and females from the same species and strain, (3) the degree of correlation in the responses of different strains within the same species, (4) the comparability of rats and mice with respect to both overall sensitivity and preferred target organ, (5) the comparability of rodents and other species, particularly long-lived species such as primates, (6) the comparison of potency indices for the same chemical administered by different routes or dosing schedules, and (7) the comparison of potency indices for lifetime and less than lifetime exposure.

METHODS EMPLOYED: An eight point set of acceptability criteria were developed for evaluating studies reported in the literature and deciding which should be abstracted into the computerized data base. Using these criteria an operational computer data system has been constructed that includes all of the NCI bioassays for which technical reports were published prior to July 1980 as well as the set of Weisberger experiments on aromatic amines. Additional data from bioassays on these same compounds that were reported in the carcinogenesis literature and that satisfied the acceptability criteria have also been incorporated into the system.

To date the data system has been used primarily for internal error checks; and the accuracy of the pathology codes, reported tumor incidences, and author's interpretations have all been verified. Statistical computer programs have also been developed for calculating relative potency indices using information from this data file in order to address the various issues outlined in the above stated objectives. (A series of papers which describes the contractor's index of carcinogenic potency and their conventions for calculating the TD_{50} empirically are in preparation.)

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Given our increasing reliance on laboratory animal data to generate quantative estimates of human cancer risk, there is a clear need to improve our ability to extrapolate cancer screening results across species. Furthermore, the construction of the proposed data base will provide scientists interested in risk estimation with a valuable new research resource.

PROGRAM RESOURCES, INC. - Rockville, MD 20852
(NO1-CP-15733-74)

TITLE: Statistical Analysis of Bioassay Data

CONTRACTOR'S PROJECT DIRECTOR: Dr. Jim Joiner

PROJECT OFFICER (NIEHS): Dr. Joseph K. Haseman
Research Mathematical Statistician
Statistics and Biomathematics Branch, BRAP

DATE CONTRACT INITIATED: July 31, 1981

CURRENT ANNUAL LEVEL: $195,932

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this contract are to provide statistical and computational expertise and resources to summarize, analyze, and aid in the interpretation of data from various NTP experiments. These investigations consist of the carcinogenesis bioassay, pre-chronic studies, and certain other "special studies" with laboratory animals.

METHODS EMPLOYED: During the first year of this contract, statistical analyses have focused primarily on data from subchronic studies. These data are abstracted from reports prepared by a number of NTP contract laboratories (each summarizing results for a particular chemical under test). The variables of interest include organ and body weights, hematology and clinical chemistry parameters, and histopathology findings. For each chemical the data are computerized, pairwise comparisons and trend tests carried out, and summary statistical reports prepared. This information is then used for the selection of doses for the chronic study. Although little chronic study data (e.g., tumor incidence rates) have been analyzed under this contract to date, it is anticipated that during the next year these data will constitute an increasing proportion of the overall workload.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE NTP: This contract provides the statistical support necessary to effectively analyze large volumes of pre-chronic and chronic data generated from various NTP laboratory animal studies.

DYNAMAC CORPORATION - Rockville, MD 20852
(NO1-ES-2-8001)

TITLE: National Toxicology Program Computer Support

CONTRACTOR'S PROJECT DIRECTOR: Ms. Nancy Bonney

PROJECT OFFICER (NIEHS): Mr. R. M. Rowley, Computer Systems Analyst, Computer Technology Branch, BRAP

DATE CONTRACT INITIATED: January 1, 1982

CURRENT ANNUAL LEVEL: $925,243

PROJECT DESCRIPTION

OBJECTIVES: The major objective of this contract is to provide the data entry, computer programming, and coding support required to produce technical reports for the NTP Animal Bioassay Program. Secondary objectives include the provision of data entry validation and report generation for NTP Bioassay Program Management Tracking systems.

METHODS EMPLOYED: The standard nomenclature of the Pathology Code Dictionary is used to code tumor diagnoses for rats, mice and hamsters in all completed bioassays. These codes are then keyed onto computer files on the DCRT computer. Computer programs are then run against the data to produce pathology tables and statistical reports which are used in the NTP Technical Reports.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE NTP: The Animal Bioassay Program is the major testing program of the NTP and is responsible for determining the carcinogenicity/toxicity of hundreds of chemical compounds to which the population of the U.S. are exposed annually. The Technical Reports on these chemicals are the official NTP publication describing the results of this testing. The pathology and statistical tables produced by the contractor make up the majority of the reports and are the single source for determining the carcinogenicity/toxicity of the chemical.

EPIDEMIOLOGY BRANCH

EPIDEMIOLOGY BRANCH
Summary Statement

The major ongoing field investigation within the Epidemiology Branch is the Breast Milk and Formula Project, a prospective, birth cohort study of about 900 North Carolina children. Clinical data on growth, morbidity, and development are gathered on the children; levels of widespread contaminant chemicals, such as polychlorinated biphenyls and DDE (the stored metabolite of DDT) are measured in the mother at birth and in breast milk over time. Enrollment of children has been completed; a substantial number of the cohort is now two to three years old; most of the chemical analyses are complete, and data analysis has begun. The very high prevalence of breast milk contaminated with these chemicals (more than 90%) has been confirmed; and data on the association between duration of breast feeding and contaminant level have been evaluated.

A laboratory program has been initiated to develop assays generating sensitive measures of the effects of environmental exposures on human populations, and to use these for monitoring human populations in epidemiologic studies. Measures will be developed that could indicate exposure to environmental toxins (such as the presence on mutagenic substances in body fluids), alterations in metabolism caused by exposure to toxic substances (such as the effects of PCB's and other agents on the activity of placental enzymes), and outcomes at a cellular and molecular level (such as somatic cell mutations) that may indicate increased risk of an adverse health effect. The development and validation of assays will be integrated with their use in specific epidemiologic studies of environmental factors.

The reproductive epidemiology program emphasizes the development of new methods for measuring and analyzing human reproductive outcomes, particularly fertility, sub-clinical early fetal loss, spontaneous abortion, fetal growth, and birth weight. Applied problems of measuring early fetal loss are being pursued in a joint project with NICHD. A recently-developed assay for human chorionic gonadotropin will be used to monitor pregnancies in conceptable women. A study of the validity of measuring spontaneous abortion risk by interview is being conducted. A number of more theoretical issues are also being addressed by means of mathematical modeling and simulation studies.

Demographic studies, using Vital Statistics data and indirect exposure assessments, have continued. This year, a number of new studies in this area were begun and analysis has been completed on three projects. Data collection for a case-control study of adult cancer risk in relation to maternal smoking during pregnancy has been completed. Preliminary analysis has focused on the quality of these data which were obtained from both study subjects and their parents. Another case-control study, which was concerned with the possible association between selenium and other trace elements and skin cancer, has been completed.

A program emphasis on environmental causes of chronic renal failure is being developed. A protocol for a case-control comparison of cases with renal failure and population controls is planned. Preliminary data collection and identification of cases is expected to begin before the end of the year. The feasibility of several related projects, including retrospective follow-up of childhood lead poisoning cases, is being investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43001-10 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Demographic Investigations of Potential Human Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dale P. Sandler	Staff Fellow	EB	NIEHS
Other:	David G. Hoel	Program Director	BRAP	NIEHS
	Carl A. Keller	Epidemiologist	EB	NIEHS

COOPERATING UNITS (if any) Statistics and Biomathematics Branch, NIEHS; Division of Epidemiology and Statistics, Radiation Effects Research Foundation; Department of Family and Preventive Medicine, University of Southern California Medical School

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of demographic investigations. Current research activities include a correlational analysis of suspected liver cancer risk factors and liver cancer mortality in the United States, a death certificate analysis of occupation and lung cancer mortality, and an analysis of secular trends in the distributions of breast cancer risk factors in Hiroshima and Nagasaki, Japan. In addition, the influence of environmental factors on mortality from renal cancer and chronic renal failure is being explored using mortality data for U.S. counties, census data, and data from the NIOSH occupational hazards survey. Other demographic studies are also being initiated.

PROJECT DESCRIPTION

METHODS EMPLOYED: Potential environmental health hazards are studied using available demographic vital statistics and other population data. Specific analyses conducted include (1) analysis of time trends and geographic patterns of primary liver cancer mortality in the U.S.; (2) analysis of the association between usual occupation and death from lung cancer in Alameda County, California; (3) analysis of secular trends in the distributions of breast cancer risk factors in Hiroshima and Nagasaki, Japan; (4) analysis of renal cancer mortality in the U.S. counties where a significant proportion of the population is employed in lead related industries; (5) analysis of occupational and demographic factors associated with mortality from chronic renal failure in U.S. counties; (6) analysis of the association between percent foreign born and cancer mortality in U.S. counties; and (7) analysis of the relationship between natural fluoride levels in drinking water and hospital discharges for hip and other fractures among the elderly.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Age-adjusted liver cancer mortality has been stable over time for all but non-white males for whom there has been a small but significant time-related increase in mortality. Time trends and race and sex differences parallel trends seen for cirrhosis mortality, but an excess mortality for white males in two Southern U.S. regions points to other environmental risk factor(s). (2) Using a case-control approach, odds ratios were calculated for employment in specific occupations to compare 'cases' who died from lung cancer to 'controls' who died from other cancers. Previously reported excess risks for individuals employed as electricians, painters, plasterers, construction workers and bus and truck drivers was confirmed. Two additional occupations not previously reported, aircraft mechanics and machine operators, were also associated with excess lung cancer risk. (3) The results of a large mail survey sent to atomic bomb survivors in Hiroshima and Nagasaki has been studied with respect to menarche, first birth, menopause and weight. Age at menarche was found to decrease from 16.4 to 14.4 years from 1902 to 1942, although a temporary increase in age at menarche was seen during the 1940's war years. Breast cancer risk factors among Japanese and U.S. women were compared in an attempt to understand differences in breast cancer risk between the two countries. (4) An association between renal cancer mortality and employment in lead-related industries could not be demonstrated using an ecological approach. This may well be due to the small number of individuals in each county actually exposed to lead rather than due to the lack of such an effect. Work on items (5), (6) and (7) will continue through the next fiscal year. Appropriate data sets have been identified and collected and preliminary analyses have been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Correlational analysis of large data sets leads to the generation of hypotheses concerning environmental exposures and disease risk. Identifying factors associated with disease risk or changes in risk patterns may lead to an understanding of the etiology of some major chronic diseases.

PUBLICATION

Milne, K.L., Sandler, D.P., Everson, R.B., and Brown, S.M.: Lung cancer and occupation in Alameda County: A death certificate case-control study. Am. J. Industrial Medicine, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43002-06 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Breast Milk and Formula Project

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Walter J. Rogan	Medical Officer	EB	NIEHS
Other:	Beth C. Gladen	Statistician	SBB	NIEHS
	James D. McKinney	Chief	LEC	NIEHS
	Douglas B. Walters	Chemist	LEC	NIEHS
	Richard B. Everson	Epidemiologist	EB	NIEHS
	Thomas K. Wong	Staff Fellow	EB	NIEHS

COOPERATING UNITS (if any) Statistics and Biomathematics Branch, Laboratory of Environmental Chemistry, NIEHS; Raltech, Inc., Madison, WI; Science Applications, LaJolla, CA; Wake Area Health Education Center, Raleigh, NC; Durham Women's Clinic, Durham, NC; East Carolina School of Medicine, Greenville, NC

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polychlorinated biphenyl (PCB) contamination of breast milk in the ppb range is well documented, but health effects on infants fed such milk are unstudied. This project involves: (1) establishment of a cohort of breast and formula fed infants; (2) development of methodology to obtain reliable and reproducible samples of human body fluids and tissue from mother and child; (3) development of reliable methods for analysis of PCB's and other related chemicals; (4) development and application of statistical procedures for the analysis of data generated from the study; and (5) evaluation of the children for specific outcomes thought to be related to organochlorine exposure including PCB's.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is an ongoing prospective, or follow-up study. Field personnel were hired, trained in protocol administration, and situated at participating hospitals. Subjects were enrolled at the various hospitals, informed consent was obtained, and a questionnaire administered to each mother at approximately the time of delivery. In addition, samples of breast milk, formula, colostrum placenta, and maternal blood were collected from each subject.

These samples are subjected to gas chromatographic and neutron activation analysis for PCB's DDE(1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane), total organic and chlorine (TOCl) and bromine (TOBr), and total soluble organic chlorine (TSOCl) and bromine (TSOBr) in the ppb range. Some of the specimens of placental tissue will also be tested for levels of mixed function oxidase enzymatic activity.

The children are examined, and follow-up appointments made. Serial examinations including behavioral evaluations are performed at birth, 6, 12, 18, 24 months, and then yearly over the first years of the child's life.

MAJOR FINDINGS AND PROPOSED COURSE: Data analysis is continuing. Data collection in North Carolina will continue. Major progress to date has been enrollment of all 900 participants and the perfection of techniques required for collection of samples without possibility of contamination. Quality assurance (QA) procedures for control of chemical analyses and the development of methodology for reliable and reproducible ppb level determination of PCB, DDE, TOCl and TOBr have been completed for milk and blood. Similar QA work is continuing for formula and placenta (TSOBr and TSOCl only). Analysis of complete sets of mothers' samples has been initiated. Initial results indicate that PCB and DDE levels decline over time spent lactating, and that women with higher levels of DDE breast feed for shorter lengths of time. Maternal blood samples have higher levels than cord blood or placenta.

The remaining objectives are: (1) to investigate the relationship between PCB and TOCl levels in the neonate and a number of specific outcomes; (2) to follow breast-fed and non-breast-fed infants over a period of years and look for differences in incidence of a number of specific outcomes; (3) to generate other hypotheses about toxic effects of chronic low dose PCB exposure in children; (4) to establish a cohort of children for follow-up studies; and (5) to determine whether there is an association between PCB or TOCl levels and placental mixed function oxidase activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health effects of these low dose environmental pollutants are not well-studied in children, and this project should allow identification and quantification of those that occur short term in this group. The methodology for studying such phenomenon is also of interest, and the development of a field efficient method for investigating low level pollutants, such as PCB's, in humans is important.

PUBLICATIONS

Rogan, W.J., and Gladen, B.C.: Monitoring breast milk contamination to detect hazards from waste disposal. Environmental Health Perspectives, in press.

Rogan, W.J.: Epidemiology of fat soluble contaminants in the food chain. In Miller, R.W., and Finberg, L., (Eds.): Chemical and Radiation Hazards to Children., in press.

Rogan, W.J.: PCBs and cola-colored babies: Japan 1968, Taiwan 1979. Teratology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43003-04 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies in Pediatric Lead Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Walter J. Rogan	Medical Officer	EB NIEHS
Other:	Beth C. Gladen	Statistician	SBB NIEHS

COOPERATING UNITS (if any)

Statistics and Biomathematics Branch, Medical University of South Carolina

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lead Exposure and undue absorption occurs not only in inner city children exposed to paint but also in children around smelters and those whose parents work with lead. Although the encephalopathy and renal disease of acute lead intoxication are well described, other toxicities of lead as well as factors that alter susceptibility need further research. Investigation into genetic differences in susceptibility to the effects of lead on blood formation has been initiated.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: A study of genetic variability in response to the toxicity of lead on blood formation has been designed. As part of the study plan data collection was performed on contract. The response variable is erythrocyte protoporphyrin (EP), the immediate precursor to heme in blood formation. Blood lead level is the exposure variable, and the amount and activity of amino levulenate dehydrase (ALA-D) the genetic marker. Children with known high or low responses of EP at a given lead level were selected from a prescreened population. They were revisited, and their blood lead, EP, and ALA-D levels determined. Approximately 200 hyper responders and 200 hypo responders were tested. The hypothesis under investigation is that children who differ in their EP response will also differ in their ALA-D level.

MAJOR FINDINGS AND PROPOSED COURSE: The Medical University of South Carolina was identified as a suitable data source, and records on the 6000 or so prescreened children in their program were obtained. A stratified sample for revisit was constructed, and co-investigators at the medical school contacted the children and conducted the blood work. Data gathering was completed in the summer of 1982, and analysis has been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Lead is an ubiquitous pollutant, and children are most vulnerable to it. Lead absorption can be both prevented and treated. Thus, an understanding of its human toxicity is appropriately within the NIEHS mission. Current screening programs continue to find and treat children with undue lead absorption. The decision to treat is made on the basis of laboratory tests rather than clinical illness, and thus it is likely that some children are treated unnecessarily. Successful identification of children with greater susceptibility would allow more effective screening for children likely to show toxicity.

PUBLICATIONS

Rogan, W.J.: Some practical problems and solutions in lead poisoning prevention programs. In Chisolm, J.J., Jr. and O'Hara, D.M., (Eds.): Lead Absorption in Children. Baltimore, Urban and Schwarzenberg, 1982, pp. 201-210.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43004-04 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Environmental Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dale P. Sandler	Staff Fellow	EB	NIEHS
Other:	Richard B. Everson	Epidemiologist	EB	NIEHS
	Walter J. Rogan	Medical Officer	EB	NIEHS
	Allen J. Wilcox	Medical Officer (Research)	EB	NIEHS

COOPERATING UNITS (if any) School of Public Health and Memorial Hospital, University of N.C., Chapel Hill; Wilson Dermatology Clinic, Wilson, N.C.; Food and Drug Administration; Center for Disease Control

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves a number of studies of chronic disease or cancer epidemiology. It includes a preliminary investigation into the etiology of Reye's Syndrome; an investigation into the relationship of maternal smoking and subsequent cancer; an investigation into the relationship of selenium and other trace elements to skin cancer; and an investigation into the relationship of environmental factors and chronic renal failure.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) Collaborative arrangements with two other agencies (CDC, FDA) have been established for the collection and analysis of tissue samples from children dying from Reye's Syndrome or other liver diseases. Aflatoxin B levels will be measured in order to determine if there is an association between Aflatoxin B exposure and pathologically confirmed Reye's Syndrome. (2) An epidemiologic study of the relationship of trace elements, particularly selenium, to the occurrence of skin cancer has been completed in Wilson, North Carolina. Clinical data from patient examinations, historical data from questionnaires, and laboratory data from blood samples were collected and analyzed to test the hypothesis of an inverse relationship between selenium and skin cancer. The interaction of other trace metals and vitamins was also studied. Patients with low selenium levels had a consistent increase in risk of skin cancer as compared with two control groups. The association was strongest in young patients with little sun damage. Vitamin A was also suggested as a protective factor against skin cancer. (3) A case-control study of adult cancer risk and maternal smoking during pregnancy has been conducted using cancer patients from UNC Memorial Hospital and both randomly selected controls and controls identified as "friends" of cases. Data collection has been completed: 520 case-control pairs were interviewed as were 744 relatives of the 1040 cases and controls. Preliminary results indicate that individuals can provide adequate smoking histories for their parents. Analysis related to the study hypothesis has just begun. (4) A proposal to study environmental factors and chronic renal failure has been developed. Using a case-control design, the study will compare the frequency of exposure to a variety of factors among cases with renal failure and among population controls without renal disease. Exposures of interest will include lead, cadmium, and mercury exposure, analgesic consumption and history of past streptococcal and urinary tract infections. (5) The feasibility of long-term (retrospective) follow-up of childhood lead poisoning cases is also being explored.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Standard epidemiologic methods have been useful in understanding etiologic agents in chronic diseases. The standard methods of epidemiology have, however, not been used extensively in studies of environmental chemicals and human disease other than cancer. These studies all examine fairly wide-spread exposures that have known or suspected association with disease in humans. Documentation of these kinds of associations allows preventive intervention or risk modifications by decreasing exposure. Since the exposures studied here are common, the potential for significant disease reduction seems high.

PUBLICATIONS

Everson, R.B., Byar, D.P., and Bischoff, A.J.: Estrogen predisposes to cholecystectomy but not to stones. Gastroenterology 82: 4-8, 1982.

Sandler, D.P., Comstock, G.W., and Matanoski, G.M.: Neoplasms following childhood radium irradiation of the nasopharynx. J. Natl. Cancer Inst. 68: 3-8, 1982.

Rogan, W.J.: Pesticides and PCBs in monitoring for environmental risk. Annual Reviews in Public Health, in press.

Rogan, W.J.: Environmental and Occupational Medicine. In Arnold, C., (Ed.): Advances in Disease Prevention, Volume I. New York, Springer, 1981, pp. 272-298.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43007-03 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Detection of human exposure to mutagenic substances by analysis of body fluids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard B. Everson Epidemiologist EB NIEHS

COOPERATING UNITS (if any)
National Institute of Occupational Safety and Health; Department of Medicine, University of North Carolina, Chapel Hill, N.C.

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is to develop methods for detecting mutagenic substances in human body fluids and to use these methods in studies of environmental epidemiology. Laboratory methods necessary for these studies are being further investigated and body fluids from individuals suspected to have occupational or other environmental exposure to mutagenic substances analyzed to confirm and partially quantitate such exposure.

PROJECT DESCRIPTION

METHODS EMPLOYED: This study involves two overlapping phases: assay development and field studies. Assay development will adapt short term mutagenesis assays to the measurement of mutagenic substances in body fluids. Such adaptation will include investigation of (1) methods for sample extraction, concentration and deconjugation; (2) sensitivity of these assays and their response to interactions between elements of complex biologic mixture; (3) possible technical artifacts and statistical interpretation of results. Initial work will focus on using the Salmonella Plate Assay, but the efficacy of other endpoints such as 8-azaguanine resistance in bacteria will be investigated. In some instances chemical determinations for specific substances will be employed so that these determinations can be correlated with mutagenesis experiments. In addition, investigation of the most effective methods for timing, collection, storing, and processing samples from human subjects will be conducted by analysis of urine from patients undergoing chemotherapy for malignant diseases. Results from these investigations will be used in the design and interpretation of studies of other human populations.

MAJOR FINDINGS AND PROPOSED COURSE: Two studies have been completed. A preliminary survey of subjects with and at risk for carcinoma of the bilharzial bladder found no evidence of mutagenic substances in urine samples, but required development and application of a procedure for quantitating bacteria in the plate lawn to prevent falsely positive interpretation of assay results. A second study did not confirm a recent report indicating that mutagenic substances are present in urine samples from non-smokers with cirrhosis. Our studies suggested the earlier findings may have been due to technical interference with the testing procedure by non-mutagenic materials in urine. Data is currently being analyzed from assays of urine samples from nurses and pharmacists who mix or administer cancer chemotherapy. This study is being done in collaboration with researchers at NIOSH. We plan to continue work on the development of a semi-automated system for quantitating the bacterial lawn in the mutagenesis tests used for these studies and are currently analyzing samples of amniotic fluid for the presence of mutagenic substances.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The assay of human body fluids for the presence of mutagenic substances should provide a means of detecting human exposure to genotoxic agents in the environment. The short term bioassays employed in these studies are capable of identifying the presence of many different mutagenic substances. Accordingly, such monitoring could both detect unanticipated mutagenic substances or their metabolites and monitor known or suspected exposures at least semiquantitatively. These capabilities should aid in the detection and evaluation of human exposure to mutagenic substances.

PUBLICATIONS

Everson, R.B., Gad-el-Mawla, N.M., Attia, M.A.M., Chevlen, E.M., Thorgeirsson, S.S, Alexander, L.A., Flack, P.M., Staiano, N., and Ziegler, J.L.: Analysis of human urine for mutagens associated with Carcinoma of the bilharzial bladder by the Ames Salmonella Plate Assay: Interpretation employing quantitation of viable lawn bacteria. Cancer, in press.

Everson, R.B., Flack, P.M., and Sandler, R.S.: Urinary excretion of mutagens in cirrhosis: Limited evidence of an association. Environmental Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43008-03 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Use of the Laboratory in studies of environmental epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard B. Everson	Epidemiologist	EB	NIEHS
Other:	Thomas K. Wong	Staff Fellow	EB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	1.4	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long term objective of this project is the effective use of the laboratory in studies of environmental epidemiology. It will include interdisciplinary development of ideas and methodologies, coupled with attention to details of both the laboratory procedures and the gathering and analysis of data concerning human subjects. The effort will focus on the development of techniques for identifying genetic damage and alterations in metabolism associated with human exposure to potentially toxic substances, and use of these techniques in epidemiologic studies.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: To encourage an interdisciplinary approach to studies of human disease etiology, especially disease related to genetic toxicology, a laboratory unit has been established within the Epidemiology Branch of the Biometry and Risk Assessment Program. The laboratory is currently using bacterial mutagenesis assays to detect the presence of mutagenic substances in human body fluids (Z01 ES 43007-03 EB), assays for mixed function oxidase enzymes in human placenta to assess possible alterations in enzyme function associated with organo-chlorine pollutants (as part of project Z01 ES 43002-06 EB), and an assay of human lymphocytes designed to use resistance to 6-Thioguanine to detect mutation occurring in vivo.

MAJOR FINDINGS AND PROPOSED COURSE: Findings for bacterial mutagenesis assays of human body fluids are discussed elsewhere (Z01 ES 43007-03 EB). Nearly 100 placental samples have been assayed for aryl hydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase activity. As previously observed, placental samples from women who smoked during pregnancy showed greatly elevated levels for both these enzymes; results will be further analyzed when data on organochloride pollutant levels become available. Use of similar assays for studying effects of human exposure to pyrolysis products on placental enzymatic function is planned. Investigation of the assay for in vivo mutation revealed the absence of a genetic basis to resistance to 6-Thioguanine for most cells scored as resistant under the conditions initially proposed in the literature for this assay. We plan to investigate the affect of alternative conditions on this assay. We have also started work on an assay for DNA strand breaks. We plan to establish assays for detecting genetic damage in man and use these initially to study effects of well-documented exposures on these assays.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Disease is the endproduct of interactions between host susceptibility and environmental exposures which proceed by a biologic mechanism. In the past, the laboratory has been of great help in defining each of these factors (susceptibility, exposure, mechanism, and outcome) in studies of infectious disease. In recent years, development of laboratory systems for measuring certain aspects of each of these factors, as they relate to the chronic diseases, has been rapid and exciting, especially in the area of genetic toxicology. Currently or in the near future, it may be anticipated that capabilities will exist to measure exposures to xenobiotics in ppb range or better, to classify genetic susceptibility by DNA repair capabilities, to seek biochemical mechanisms for events now related only phenomenologically, and to assess risk by observing direct effects on DNA or somatic cell mutation.

Applications of these tests to human populations, however, will be a difficult and complex undertaking. Test validation will be necessary, both in terms of its biologic meaning and of the more traditional biostatistical concepts of sensitivity and specificity. Details of both the laboratory procedures employed and subjects tested will require equivalent attention, preferably by scientists or groups of scientists with inter-disciplinary backgrounds and an understanding of both the

test and the populations tested. Many factors concerning the subjects tested will require consideration, including evaluation of susceptibility and past exposures other than the specific exposure under study. A program aimed at developing both laboratory methods and epidemiologic methods that use the laboratory effectively should be of great utility in this undertaking.

PUBLICATIONS

Chiu, P.-L., Wong, T.K., Fu, P.O., and Yang, S.K. 7-Methylbenzo(a)pyrene and Benzo(a)pyrene: Comparative metabolic study and mutagenicity testing in Salmonella typhimurium TA100. In: Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry. Sixth International Symposium, in press.

Wong, T.K., Chiu, P.-L., Fu, P.P., and Yang, S.K.. Metabolism and mutagenicity testing of 7-Methlbenzo(a)pyrene. In: Kolber, A., Wong, T.K., Grant, L., and Hughes, T. (Eds.): In Vitro Toxicity Testing: Current and Future Possibilities. Nato Advanced Research Institute Series. New York, Plenum Press, in press.

Everson, R.B.: Identification of population sensitive to the impact of the by-product of technology transfer. Proceedings of the Symposium of the Biomedical Impact of Technology Transfer, Cairo, Egypt, February 1980, in press.

Griesemer, R.A., Ulsamer, A.G., Arcos, J.C., Beall, J.R., Blair, A.E., Collins, T.X.F., de Serres, F.J., Everson, R.B., Gamble, J.F., Gaylor, D.W., Groth, D.H., Kang, H.K., Keenlyside, R.A., Lloyd, J.W., Nettesheim, P., Saffiotti, U., and Weisburger, E.K.: Report of the Federal Panel on Formaldehyde. Consumer and Product Safety Commission, Washington, D.C., 1980. Environmental Health Perspectives. 1982, Vol 43, pp. 139-168.

Brusick, D., de Serres, F.J., Everson, R.B., Mendelsohn, M.L., Neel, J.V., Shelby, M.D., and Waters, M.D.: Monitoring the human population for mutagenic effects: Detection of gene mutations and DNA damage. In Bloom, A.D., (Ed.): Guidelines for Studies of Human Populations Exposed to mutagenic and Reproductive Hazards. March of Dimes Birth Defects Formation, 1981, pp. 111-140.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44003-05 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Allen J. Wilcox	Medical Officer (Research)	EB	NIEHS
Other:	Beth C. Gladen	Statistician	SBB	NIEHS
	Dale P. Sandler	Staff Fellow	EB	NIEHS
	Carl A. Keller	Epidemiologist	EB	NIEHS
	Bruce C. Nisula	Medical Officer (Research)	DEB	NICHD
	Michele R. Forman	Epidemiologist	EB	NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, Epidemiology Branch,
National Institute of Child Health and Human Development

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.15	1.75	1.40

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The reproductive epidemiology program emphasizes the development of new methods for measuring and analyzing human reproductive outcomes. Such outcomes include fertility, sub-clinical early fetal loss, spontaneous abortion, fetal growth, and birth weight. Each of these outcomes is affected by environmental factors, and represents a likely endpoint for studying the effects of toxins on human reproduction. Applied problems of measuring early fetal loss are being pursued in a joint project with NICHD. A recently-developed assay for human chorionic gonadotropin will be used to monitor pregnancies in conceptable women. In another current study, the validity of measuring spontaneous abortion risk by interviewing women is being evaluated. More theoretical problems in the analysis of spontaneous abortion risk and of birth weight are being approached through the development of mathematical models. Finally, age-at-menarche, seasonality, and other factors are being studied for their effect on reproductive outcomes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Reproductive outcomes are a sensitive endpoint for the detection of human exposure to environmental hazards. The purpose of this project is to develop sound epidemiologic methods for measuring human reproductive outcomes, and to clarify the relationship of those outcomes to specified exposures. In particular, effort has focused on (1) an exploration of human fertility as an endpoint in environmental studies; (2) refinement of methods for analyzing the risk of spontaneous abortion; (3) development of new methods for analyzing the effect of environmental hazards on birthweight; (4) measurement of the validity of recall data regarding prior spontaneous abortion; (5) design of a prospective study of subclinical early fetal loss, using newly-developed assays for human chorionic gonadotropin.

MAJOR FINDINGS AND PROPOSED COURSE: (1) We have developed a model which partitions the risk of spontaneous abortion into biologically-plausible categories, and which offers a basis for developing sound analytic tools for the further study of spontaneous abortion. (2) We have measured the accuracy of spontaneous abortion recall among 400 women who have had at least one such abortion. These data will provide a benchmark for evaluating the quality of recall data in other studies of spontaneous abortion. (3) We have initiated a collaboration with NICHD for the study of very early pregnancy loss. A pilot study is in progress which is intended to be the basis for a larger study of environmental effects on very early fetal loss. (4) We have assessed the relation of age at menarche to subsequent reproductive failure, and the relation of seasonality to perinatal mortality. (5) We are analyzing data from a large study of infant mortality in a high-risk community, in collaboration with NICHD.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Human reproductive outcomes are, in theory, highly relevant endpoints for the study of environmental exposures. In practice, these outcomes are persistently difficult to measure, analyze, and interpret. This project intends to strengthen the epidemiologic tools for measuring and analyzing fertility, sub-clinical fetal loss, spontaneous abortion and birthweight, and by so doing, better assess the impact of environmental factors on human reproductive failure.

PUBLICATIONS

Wilcox, A.J., Treloar, A.E., and Sandler, D.P.: Spontaneous abortion over time: Comparing occurrence in two cohorts a generation apart. Amer. J. Epidemiol. 114: 548-553, 1981.

Wilcox, A.J.: Surveillance of pregnancy loss in human populations. Amer. J. Ind. Med., Journal Symposium on Reproductive Toxicology, D.R. Mattison, ed., in press.

Wilcox, A.J.: Quantitative effects of chemicals on fertility. Workshop on Quantitative Estimation of Risk to Human Health from Chemicals; International Program for Chemical Safety, and the Scientific Group on Methodologies for Safety Evaluation of Chemicals, in press.

STATISTICS AND BIOMATHEMATICS BRANCH

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

A variety of collaborative research projects are underway in the area of mathematical population genetics. A stochastic model has been developed that depicts the evolution of transposable elements in finite Mendelian populations. The research effort dealing with models for the study of nucleotide evolution is being continued with special attention being focused on the modification of these models to reflect the high rates of transitions to transversion observed in recent studies of mitochondrial DNA. Additional work on the modeling of the interaction of genetic recombination with DNA repair has also been initiated.

The major emphasis in risk assessment research continues to be on the modification and development of statistical procedures for using laboratory animal data to assess potential human health risk associated with exposure to hazardous environmental agents. As in the past most of the research effort has been concentrated on developing and evaluating procedures for estimating cancer risk. Branch researchers modeled the massive data base generated in the NCTR ED_{01} study of 2-AAF and demonstrated that not even the more sophisticated of the traditional time-to-tumor models provided a fully adequate fit to the experimentally observed tumor incidence data. This same data base was employed to evaluate the ability of the multistage model to depict the change in risk over time that occurred after dosing was discontinued. In both instances alternatives to the standard extrapolation procedures were proposed that yielded improved fits to the experimental observations. Research has also continued into the more biomathematical aspects of low-dose extrapolation. In order to deal with the essentially undescribed phenomenon of tumor growth following the initiation process, a model has been developed which assumes that tumor growth is a linear function of the cellular concentration of certain metabolites of the chemical carcinogen under study that are covalently bound to the DNA.

One of the most rapidly expanding efforts within the Branch has been the provision of applied statistical research support to the Toxicology Research and Testing Program of the NTP. Extensive research has been conducted on possible modifications of the NTP cancer bioassay design that would render the data it generates more useful for low-dose extrapolation without sacrificing cancer detection potential. In addition, research is being conducted on the development of statistical methodology for the utilization of historical control data in a formal testing framework and on the identification of factors responsible for intra- and inter-laboratory variability in control tumor incidence. Research also continues on the development of statistical procedures applicable to the analysis of short-term, in vitro test data. As part of this effort a major analysis has been performed on data from a large, collaborative European-US study of the role of genetic drift in observed interlaboratory variability in short-term screening test results.

The Statistics and Biomathematics Branch also provides a comprehensive statistical consulting service for the Institute's scientific staff. This effort covers a wide range of activities related to experimental design and data analysis. In the area of experimental design, problems addressed include sample size de-

termination, estimation of power, control of possible confounding factors, and optimum allocation of animals. The data analysis activities provided by the Branch include tabulation of summary statistics, curve fitting, significance testing, and interpretation of test results. These efforts are closely coordinated with the Computer Technology Branch.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40002-12 SBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Other:	Doug Simpson	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a continuing effort to develop new statistical methodology to deal with a variety of problems related to the Branch's consulting activities and collaborative research. Specific areas in which statistical research is being conducted include detection of hyper-Poisson variability when count data are not identically distributed, detection of multivariate normal outliers, and evaluation of the two main statistical analyses for 2 x 2 contingency tables.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques ranging from Monte Carlo simulation procedures to analytical test development and mathematical modeling have been employed to address various statistical methodology problems arising from the Branch's intramural consulting activities and collaborative research.

MAJOR FINDINGS AND PROPOSED COURSE: (1) For count data that are not identically distributed, e.g. in a regression context, locally optimal procedures have been derived for the detection of hyper-Poisson variability and have been shown to improve substantially upon existing statistical procedures. (2) A locally optimal test has been obtained for the detection of outliers in multivariate normal data and is applicable without a prespecification of the number of outliers anticipated. (3) Research is ongoing that compares the Fisher exact test and the two-sample binomial test with regard to the analysis of 2 x 2 contingency tables. Preliminary results indicate that the comparison is complex and heavily dependent on an investigator's untestable assumptions about the stochastic behavior of the individual dichotomous observations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of improved statistical methodology is essential if the Branch is to collaborate and consult with the Intramural Research Program effectively.

PUBLICATIONS

Schwager, S.J. and Margolin, B.H.: Detection of multivariate normal outliers. Annals of Statistics, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40004-05 SBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical Methods in Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Beth C. Gladen	Statistician	SBB	NIEHS
Other:	Takashi Yanagawa	Visiting Scientist	SBB	NIEHS
	Allen Wilcox	Medical Officer (Research)	EB	NIEHS

COOPERATING UNITS (if any)
Epidemiology Branch

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIH, NIEHS, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's activities in the field of epidemiology. The objectives are both to broaden understanding of the uses and limitations of currently employed study designs and corresponding analyses; and to develop new techniques for statistical analyses of epidemiological studies.

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques for the analysis of various kinds of epidemiological studies were devised or evaluated. Both theoretical mathematical calculations and computer simulations were used to assess techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The estimation of incidence of a disease through the use of a diagnostic test was studied. Theoretical equations were derived to show the relationship between the observed incidence and the true incidence. It was shown that even for high values of sensitivity and specificity, the two will differ markedly. These techniques were applied to the estimation of onchocerciasis rates. (2) The extent to which unmeasured confounding factors can disturb the observed odds ratio from a case-control study was assessed. The crude odds ratio was compared to the odds ratio that would have been observed had a matched study been done. It was found that, in ordinary circumstances, the effect of the confounding factor will be small. (3) Preliminary work on the effectiveness of various matching schemes in the study of spontaneous abortions was undertaken.

The proposed course is to continue research efforts on statistical methodology problems that arise in connection with the epidemiological investigations carried out by the Biometry and Risk Assessment Program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The understanding and criticism of current statistical techniques and the development of improved techniques is important for the proper evaluation of the results of epidemiological studies.

PUBLICATIONS

Wilcox, A. and Gladen, B.: Spontaneous Abortion: The role of heterogeneous risk and selective fertility. Early Human Development, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40005-05 SBB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical Analysis of Mutagenesis Testing Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Other:	Norman Kaplan	Research Mathematician	SBB	NIEHS
	Ken Risko	Mathematical Statistician	SBB	NIEHS
	Reuel Smith	Mathematical Statistician	SBB	NIEHS
	Doug Simpson	Mathematical Statistician	SBB	NIEHS
	Errol Zeiger	Head, EMTDP	CGTB	NIEHS

COOPERATING UNITS (if any)

Cellular Genetics and Toxicology Branch, TRTP

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term objective of this ongoing project remains the development of appropriate statistical techniques for the analysis of data arising from assays under study in the Environmental Mutagen Test Development Program (EMTDP). Results are also applicable to other large mutagenicity studies, such as the European Collaborative Study and the International Program for the Evaluation of Short-Term Tests for Carcinogenicity. The major focus to date has been on microbial test systems, although additional research has dealt with Drosophila. Preliminary work on in vivo and in vitro cytogenetic assays has also commenced. Statistical procedures for the design and analysis of short-term tests proposed or currently employed by other researchers in mutagenicity are assessed and new and improved procedures are developed.

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Test for mutagenicity remains the dominant assay under study, especially regarding the influence of protocol parameters upon the observed response. A major statistical analysis has been performed on the data generated by the large collaborate European-U.S. study of genetic drift as an explanation for observed substantial interlaboratory variability. A mixture of mechanistic and empirical modeling, together with statistical data analytic procedures, has constituted the main methodological approach toward the microbial systems. This also is the approach that has been adopted in dealing with the Drosophila recessive lethal and cytogenetics assays.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The set of models developed for Ames Test data provides a mutagenic index that summarizes and quantifies up to protocol uncertainties the results obtained from a single-strain Ames Test. This index lends itself to studies of response reproducibility, both within and across laboratories, and inter-strain agreement. Based on this index and related measures, the analysis of the "genetic-drift" study has reached tentative conclusions that genetic drift is not a significant factor in interlaboratory variability. (2) The analysis of an EMTDP data base on over 200 chemicals continues for the purpose of investigating sources of variability in response, with an eye toward introducing quality control procedures. (3) The Kastenbaum-Bowman statistical test, one of the most commonly employed statistical procedures in mutagenicity studies, is extremely conservative and can readily be improved upon. Tables of sample sizes necessary to achieve a specified power of detection have been prepared.

SIGNIFICANCE OF BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project has altered the ways in which mutagenicity test data are analyzed. This will result in a reduction of the percentage of false declarations of positive and negative findings resulting from use of these tests. This research effort continues to yield methodological results of interest to biometricians in numerous other areas of research.

PUBLICATIONS

Collings, B.J., Margolin, B.H., and Oehlert, G.W.: Results for binomial data, with application to the design and analysis of fluctuation tests for mutagenicity. Biometrics 37, 775-794, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41001-08 SBB

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Risk Assessment Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Hoel	Chief	SBB	NIEHS
Other:	Kenneth G. Brown	IPA	SBB	NIEHS
	Michael D. Hogan	Mathematical Statistician	SBB	NIEHS
	Norman L. Kaplan	Mathematical Statistician	SBB	NIEHS
	Marshall W. Anderson	Mathematician	LP	NIEHS

COOPERATING UNITS (if any)
Laboratory of Pharmacology; Developmental Biology Division, Health Effects Resesearch Laboratory, EPA

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the development of statistical/mathematical methodology useful in the assessment of human health risk associated with exposure to hazardous environmental agents. Special emphasis is given to the generation of improved statistical techniques for estimating potential human cancer risk from laboratory animal data. Current research efforts are particularly concerned with the role of pharmacokinetics in low-dose extrapolation modeling and with the estimation of lifetime cancer risk when the duration of exposure is less than a normal lifespan. Studies of the factors that influence species scale-up for non-carcinogenic endpoints like terata are also underway.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Since this project involves the development of statistical procedures that are applicable to risk assessment issues particularly in the area of carcinogenesis, a great deal of emphasis is given to modeling techniques. These techniques range from empirical data evaluation and curve fitting to the generation of mathematical models that attempt to approximate biological mechanisms.

MAJOR FINDINGS AND PROPOSED COURSE· The Armitage-Doll, Hartley-Sielken, and other time-to-tumor models used in low-dose extrapolation were evaluated in the framework of the NCTR ED_{01} study of 24,000 mice exposed to the known carcinogen 2-AAF. It was shown that any model that has a factorable hazard function (i.e., one that can be factored into the product of dose exclusively and a function of time exclusively) is too simplistic to accurately describe the observed tumor responses over the entire experimental range of dose and time. A modified version of the multistage model was developed and found to provide a more reasonable fit to the experimental data set.

The same data base was used to compare a "traditional" multistage model with the modified procedure in terms of their respective abilities to evaluate the change in risk over time after dosing is discontinued and to establish which stage(s) of the carcinogenic process are affected by dose. The modified version of the multistage model provided a better prediction of the results of serial dosing and a clearer impression of which stage of the carcinogenic process was affected by exposure to 2-AAF.

Research has continued on the role of pharmacokinetics in low-dose extrapolation modeling. Since little is known about the mechanism leading to tumor growth after initiation has occurred, a "black box" approach has been adopted for the modeling of this phenomenon. Specifically, it has been assumed that the probability of tumor growth is a linear function of the cellular concentration of certain metabolites of the chemical carcinogens that are covalently bound to the DNA. (One important aspect of this model is that all non-linearities are assumed to arise from the kinetic process.) The various properties of this model are now under investigation.

Research has also been initiated in the modification and development of statistical procedures useful for species scale-up in teratogenesis. Initial efforts are being concentrated on the evaluation of teratogenic data sets generated by EPA's Health Effects Research Laboratory to explore the feasibility of developing a teratogenic potency index and/or mathematical models for species extrapolation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since animal experimentation plays an ever-increasing role in the assessment of potential human health risk resulting from exposure to environmental contaminants, improved statistical/mathematical procedures for realistically assessing this risk are greatly needed.

PUBLICATIONS

Haseman, J.K., Hoel, D.G., and Jennrich, R.I.: Some practical problems arising from use of the Gamma multi-hit model for risk estimation. J. Toxicol. Environ. Hlth., 8:379-386, 1981.

Hoel, D.G.: Extrapolation of laboratory data to human health effects. In Proceedings of the Brookhaven National Laboratory Symposium on Genetic Effects of Airborne Agents, Upton, New York, February 9-11, 1981. In Environmental Science Research, 25:521-526, 1982.

Hoel, D.G.: Extrapolation models of animal toxicity data to man. In Conference Proceedings: Environmental Risk Assessment, Electric Power Research Institute 4-95 to 4-103, 1981.

Hoel, D.G.: Statistical measures of risk. In Proceedings of the Symposium on Metabolism and Pharmacokinetics of Environmental Chemicals in Man, Sarasota, Florida, June 7-12, 1981. Drug Metabolism Reviews, in press.

Peres, C.A., and Koo, J.O.: A comparison of the two-component and quadratic models to assess survival irradiated stage-7 oocytes of drosophila melanogaster. Mut. Res., 91:341-346, 1981.

Hogan, M.D. and Hoel, D.G.: Extrapolation to man. In Methods in Toxicology, edited y A. Wallace Hayes, pp. 711-731. Raven Press, New York, in press.

Hogan, M.D.: Extrapolation of animal carcinogenecity data: Limitations and pitfalls. E.H.P., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-07 SBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Other:	Charles H. Langley	Research Chemist	LAG	NIEHS
	Tom Darden	Research Mathematician	SBB	NIEHS

COOPERATING UNITS (if any)

Laboratory of Animal Genetics

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this research project is the development of mathematical models to describe phenomena encountered in population genetics. Current efforts include the development of stochastic models to describe the evolution of transposable elements in finite Mendelian populations, the continued investigation of models used to predict nucleotide substitution rates from restriction enzyme data and nucleotide sequence data, and the development of stochastic models which describe the interaction of genetic recombination with DNA repair and normal meiosis at the molecular level.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Collaborative research was conducted on problems in population genetics with scientists in the LAG. This research, which augments the population genetics studies performed by the LAG, was probabilistic in nature, emphasizing Markov chain theory and diffusion techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A stochastic model has been developed to study the evolution of transposable elements in a finite Mendelian population. An estimate of the frequency spectrum has been developed with the help of a diffusion approximation. Other aspects of the model are also under study. Particular attention has been paid to parameter estimation and the problem of nonidentifiability. Certain generalizations of the model to more than one type of transposable element are currently under study. (2) New data on mitochondrial DNA has required a more detailed analysis of the models which have been proposed to study nucleotide evolution. Efforts are currently underway to modify the models so as to accommodate the high rates of transitions to transversion that have been observed. (3) Mathematical models are being developed to describe the alkaline sucrose sedimentation patterns of parental and newly synthesized DNA. These models are necessary since the traditional analysis of such sedimentation patterns are misleading. These models are also being used to study the consequences of various hypothetical mechanisms of recombination. Future applications of these models will focus on the study of the fine structure of genetic maps.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: More realistic models for genetic phenomenon should be beneficial in predicting long-term effects of environmental changes on public health.

PUBLICATIONS

Kaplan, N.L., and Risko, K.J.: An improved method for estimating sequence divergence of DNA using restriction endonuclease mappings. J. Mol. Evol. 17:156-162, 1981.

Kaplan, N., and Risko, K.: A method for estimating rates of nucleotide substitution using DNA sequence data. Theo. Pop. Biol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 45001-02 SBB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Experimental Design and Data Analysis Methodology for Animal Bioassays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
	David G. Hoel	Chief	SBB	NIEHS
Other:				
	Christopher Portier	Mathematical Statistician	SBB	NIEHS
	Gregg E. Dinse	Staff Fellow	SBB	NIEHS
	Takashi Yanagawa	Visiting Scientist	SBB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with statistical methodological issues involved in the design and analysis of animal bioassays with particular emphasis on TRTP's two-year carcinogenesis bioassay. Current research efforts are focused on a detailed assessment of the basic experimental design employed in the TRTP cancer bioassay, on the development of statistical procedures for incorporating historical control data in a formal testing framework, and on the evaluation of various factors that may contribute to intra- and inter-laboratory variability in control tumor incidence. In addition the identification and analysis of treatment-associated patterns of change in tumor incidence has also been initiated for recently completed TRTP cancer bioassays.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The evaluation of existing experimental designs and analytical procedures and the development of new methodologies require the use of a number of different mathematical/statistical techniques including mathematical modeling, non-linear optimization, asymptotic information theory, Monte Carlo simulation, and various analytical test procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Large sample theory results showed that there is an unbalanced two-dose bioassay design that maintains cancer detection power and has optimal low-dose extrapolation properties when an underlying multistage model is assumed. However, simulation studies indicated that this design had low power for detecting an increase in cancer risk when only moderate sample sizes (i.e., 150-300 animals) are employed in a single experiment. Furthermore, increasing the sample size within the economically/logistically feasible range did not seem to alter this finding. Research in this area is being continued.

Historical control tumor incidence rates frequently show considerable inter-laboratory variation. Even within a given laboratory, there is often more variability from study to study than would be expected under a simple binomial model. Statistical methodology is being developed that will take this extra-binomial variability into account and will allow the incorporation of historical control data into a formal significance test. A related effort is underway to identify factors responsible for the extra-binomial variation in control tumor incidence. Potential sources of variation include time, laboratory, pathologist, animal supplier, and length of study.

Patterns of tumor incidence in Fischer rats have been examined in recent NTP bioassays. It was found that decreased incidences of mammary gland fibroadenomas are often correlated with decreased weight gain. Moreover, increased liver tumor incidences are frequently associated with decreased rates of leukemia/lymphoma. This investigation also indicated that extra-binomial variation in control tumor incidence rates were present in these NTP studies.

Statistical methodology development related to the utilization of historical control data will continue to be an important area of research. In addition, efforts will be made to refine the current NTP historical control tumor incidence data base. This effort includes the re-structuring of the data base itself and the development of new computer programs to summarize and analyze these data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The TRTP's carcinogenesis bioassay program is the federal government's primary means of screening compounds for carcinogenic potential. In addition it is one of the main sources of data for assessing human cancer risk. Therefore, any methodological development that improves its effectiveness is important to both the Institute and the biomedical community in general.

PUBLICATIONS

Hoel, D.G.: Conditional two-sample tests with historical controls. In Contributions to Statistics: Essays in Honor of Norman L. Johnson. North Holland Publishing Company, in press.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

During Fiscal Year 1982, the National Advisory Environmental Health Sciences Council reviewed 627 applications for which NIEHS was primary or secondary assignee. This represents an increase of 185 applications over Fiscal Year 1981. One hundred and thirty-eight new and competing awards were made: 104 regular research grants, one Environmental Health Sciences (EHS) Center and one Marine and Freshwater Biomedical (MFB) Center, three Research Career Development Awards, one Mid-Career Development Award, 18 Individual NRSAs and 10 Institutional NRSAs. These new and competing awards, plus the non-competing awards, brought the 1982 total to 421 active grants, an increase of 15 awards above Fiscal Year 1981.

Staff has continued efforts to more clearly and more precisely define the Extramural Program through the use of Program Announcements and Requests for Applications.

One Program Announcement was issued to better acquaint scientists with the NIEHS, its programs and the responsible program staff. This announcement was considered necessary because of the many inquiries EP staff had received related to review and assignment of applications by NIEHS vs. review of assignment by NIH.

A second Program Announcement, "Areas of Research Considered Peripheral to NIEHS", was issued after approval by Council. This announcement identified a number of research areas peripheral to the developing programmatic interests of NIEHS-EP and informed current and potential applicants that NIEHS would no longer accept for review or fund applications in these specific areas. Staff also prepared a memo further defining areas of decreasing interest for use by DRG Referral Officers in assignment of incoming applications, and as a reference for returning applications not relevant to NIEHS or other BIDs.

With the approval of Council, and in line with this Program Announcement, funded research grants in these areas are being phased out or have been transferred to other BIDs or funding agency. Each affected grantee has been informed of Council/Staff decisions and staff has assisted applicants in funding new sources of support or in redirecting their research efforts.

A third Program Announcement, "Environmental Medicine - Development of Diagnostic Technology for Use in Populations Exposed to Hazardous Chemicals, Particularly From Waste Dumps", was issued in an effort to obtain additional applications in order to evaluate or refine established or newly developed tests with prognostic characteristics in order to assess the effects of exposures that may occur in occupational settings, therapeutic regimes or unexpected episodes of chemical exposure.

A joint NIH announcement was issued in May reannouncing the New Investigator Research Award and identifying responsible staff and programmatic interest of each BID. Although staff has encouraged young investigators to apply for the NIRA, the number of NIRA awards continues to decline.

A single RFA was issued in '82 entitled "Molecular Genetics of Chemically-Induced Mutations in Mammalian Cells", which sought to generate interest in research to study the structure, organization and regulation of genes and the mechanisms of their alteration by chemical mutagens for development of systems for detection of populations at risk from exposure to hazardous chemicals.

A new development-type program, the Clinical Investigator (Development) Award (CIA), was approved by Council and applications will be accepted for review in FY '83. This program was developed to encourage young clinicians with interests in Environmental Health research to apply for salary and research support (up to five years) while developing research and clinical expertise under an established investigator in Environmental Health research and following training, develop clinical research within their respective institutions.

Program Analysis Unit (PAU) staff has been trained to use CRISP, IMPAC, and WYLBUR, as well as the NIEHS InteRIM system, in preparation of reports on NIEHS-EP research. EP staff has initiated discussions with other interested NIEHS staff for the development of a more sophisticated and responsive automated data system which will be used with a more detailed coding of the EP grants.

The Environmental Health Sciences Review Committee held its first meeting on April 28 and 29 as a Chartered Committee. With the appointment of this Committee, EP has assumed responsibility for review of Program Project applications and of Research Contract Proposals. It is anticipated that the workload of this committee will increase as contract review procedures are finalized. The committee will also review applications submitted in response to RFAs in which highly specialized environmental health research is identified.

RESEARCH MANPOWER DEVELOPMENT CENTER

Training Programs

The NIEHS Extramural Training Programs support pre- and postdoctoral research training in the fields of environmental toxicology, environmental pathology, environmental epidemiology and biostatistics, and environmental mutagenesis. Each of these programs has as its goal to provide appropriately trained manpower for careers in research pursuant to the missions of the Institute. Program descriptions and summaries are provided in the following text.

Environmental Toxicology

Training in environmental toxicology is focused on developing individuals for research careers pursuing an understanding of the pharmacological principles which determine the mechanism, site, and severity of damage to tissues by environmental agents. Research training focuses upon the processes by which environmental agents affect biological systems and by which biological systems influence the action and fate of these agents (metabolism, tissue distribution, etc.). Approximately 65% of our training support dollars are spent in the area of environmental toxicology.

To better define "environmental toxicology" let us divide working toxicologists into the following classes:

1. Clinical Toxicologists who deal with acute intoxication by poisons. These individuals determine causes of intoxication (which chemical?) and prescribe methods of treatment. They are usually associated with hospitals or poison control centers.

2. Experimental Toxicologists who are involved in the demonstration of safety or hazard of exposure to chemical or physical agents such as environmental chemicals or drugs. These individuals do testing on experimental models. They design protocols and analyze results. Their work usually culminates with a "safety evaluation" report including protocol descriptions and the data obtained in support of a conclusion on hazard level resulting from exposure to the particular agent.

3. Predictive Toxicologists who speculate on the hazard to man from exposure to chemical or physical agents. These individuals attempt to obtain knowledge about the pharmacodynamics and pharmacokinetics, mechanisms of action, dose/response relationships, comparative metabolism, etc. of the particular chemical involved. They do research on experimental models in an attempt to extrapolate this knowledge to better assess the hazards to man of exposure to the chemical.

4. Forensic Toxicologists who determine cause/effect relationships between poisons and pathologies. These individuals usually work with law enforcement agencies.

The NIEHS supports the training of pre- and postdoctoral students in the second and third classes of toxicologists, focusing on those individuals who wish to study the effects of environmental agents.

There are generally two schools of thought regarding how individuals should be trained for research careers in environmental toxicology. The philosophy of the first school is that a student must initially be trained as a scientist in a classical discipline and then receive additional training and experience to specialize in toxicology. This has been the general pathway by which today's established toxicologists have moved into the field. Individuals in this school argue that toxicologists must first be trained as good basic scientists so that the scientific method can be applied in well-defined experiments to determine the nature of the toxicity at the biochemical, physiological, cellular or tissue level.

The philosophy of the second school is that toxicology is a discipline unto itself which draws skills and backgrounds from various facets of the basic sciences. Therefore, a toxicologist should be trained not only by taking courses in toxicology but also in special topics which emphasize the interaction of environmental chemicals with the biochemical systems, organelles, macromolecules, or the tissue component.

The NIEHS Extramural Training Program in Toxicology supports institutions training individuals under both these philosophies. Therefore, our award portfolio is comprised of grantees in a variety of departments such as biochemistry, physiology, nutrition and food sciences, etc., in addition to Departments of Toxicology.

It is becoming quite apparent that there are needs for toxicological specialists to engage in two types of activities. The immediate need is for toxicologists to perform safety evaluations to provide data required by the regulatory agencies upon which societal decisions can be made as to whether or not the general population should be exposed to a certain material. These are the experimental toxicologists referred to above. Likewise, there is an equal demand for individuals who will advance the state-of-the-art of the science of toxicology by furthering our understanding of toxicological mechanisms of action by developing new testing methodologies and by producing new knowledge about the effects of chemicals on organisms. This new knowledge will then allow safety evaluation procedures to become more precise and more capable of predicting the true hazards of these agents in the real world.

In the 1981-82 academic year, the NIEHS supported 39 postdoctoral fellows in toxicology and 28 Institutional Training Grants in toxicology (comprised of about 190 pre- and 100 postdoctoral trainees) as follows:

Institution	Director
University of Wisconsin	Dr. Ronald Hinsdill
Massachusetts Institute of Technology	Dr. Gerald Wogan
University of Rochester	Dr. Victor G. Laties
Vanderbilt University School of Medicine	Dr. Peter Guengerich
Purdue University	Dr. John E. Christian
Medical College of Wisconsin	Dr. James Fujimoto
University of Mississippi Medical Center	Dr. Harihara Mehendale
North Carolina State University	Dr. Frank E. Guthrie
Children's Hospital Research Foundation	Dr. Ernest Zimmerman
Cornell University	Dr. Christopher Wilkinson
Roswell Park Memorial Institute	Dr. Harold Box
Albany Medical College	Dr. Rejender Abraham
University of California, Davis	Dr. James N. Seiber

Oregon State University	Dr. Ian J. Tinsley
University of Michigan	Dr. Herbert Cornish
New York University	Dr. Morton Lippmann
Johns Hopkins University	Dr. Robert J. Rubin
	Dr. Zoltan Annau
University of Cincinnati	Dr. Paul B. Hammond
Michigan State University	Dr. J. Justin McCormick
University of Kansas Medical Center	Dr. Curtis D. Klaassen
Thomas Jefferson University	Dr. C. Paul Bianchi
Yale University	Dr. Margaret Hitchcock
Medical College of Virginia	Dr. Albert Munson
University of Texas Health Sciences Center	Dr. Sheldon Murphy
University of Arizona	Dr. J. Wesley Clayton
Utah State University	Dr. Joseph Street
Dartmouth College	Dr. Roger Smith
New York University Medical Center	Dr. Roy E. Albert

Environmental Pathology

Trainees in this area focus their research on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees hold professional or academic degrees which qualify them for advanced training in gross and histopathological research dealing with the structural and functional alterations of tissues exposed to environmental chemicals. Usually training is focused on morphology, but training in the biochemical basis of environmental disease pathogenesis may also be included in these programs; and the tools of the toxicologist can also be used to elucidate the mechanistic bases of environmental diseases. Eight institutional awards supporting 23 pre-and 42 postdoctoral trainees were supported in this 1981-82 academic year, as follows:

Institution	Director
Mount Sinai School of Medicine	Dr. William Nicholson
University of North Carolina	Dr. Joseph Grisham
Duke University School of Medicine	Dr. William S. Lynn
University of Washington, Seattle	Dr. N. Karle Mottet
University of California, Davis	Dr. Donald Dungworth
Washington University, St. Louis	Dr. Michael Lieberman
State University of New York, Stony Brook	Dr. Philip Kane
University of California	Dr. Edward Smuckler

The objective of the pathologist is to determine the cause of the observed lesion, whereas the objective of the toxicologist is to assess the effects of exposure to a known agent. Nevertheless, the tools used by both disciplines in the various facets of investigation may be identical. Therefore, graduates of the environmental pathology training programs can become members of research teams involved in chemical risk evaluation utilizing laboratory animals as experimental models. Although these graduates are capable of histopathological evaluation, their backgrounds allow them to become more active members of research teams involved in chemical risk evaluation or to become independent investigators.

Environmental Epidemiology and Biostatistics

Trainees in environmental epidemiology are taught to utilize statistical and mathematical tools to assist in the identification of environmental diseases in human

populations. Training stresses non-infectious disease epidemiology with emphasis on the identification of causes of environmental diseases. The major difference between training in environmental epidemiology and infectious disease epidemiology is that human populations are studied with respect to the effects of established exposures to environmental agents, whereas epidemiological studies of infectious diseases usually begin with a pathological observation.

Biostatistics trainees learn and apply mathematical and statistical tools in assisting environmental health scientists in experimental design and interpretation. Mathematical modeling for human risk assessment based on laboratory experiments is studied. Methodologies for extrapolating the results of high dose exposures to environmental agents to predicted effects or real-world low dose exposures are also developed. Upon completion of their training, these individuals undertake research activities supportive of the development of laws and standards governing human exposures to environmental agents.

Eight institutional awards in these fields were supported by the NIEHS during the 1981-82 academic year, training 59 pre- and 17 postdoctoral students. The awards in these two areas went to the following institutions:

Institution	Director
University of North Carolina	Dr. Lawrence Kupper
University of North Carolina	Dr. Herman Tyroler
University of California, Los Angeles	Dr. Roger Detels
University of Cincinnati	Dr. Ralph Buncher
Harvard University	Dr. Richard Monson
University of Illinois	Dr. Paul S. Levy
University of Pennsylvania	Dr. Jonathan Amsel
Yale University School of Medicine	Dr. Jan Stolwijk

Environmental Mutagenesis

Trainees in this area are taught to apply the basic principles of genetics to applied studies aimed at assessing the potential genetic hazard to man of environmental chemicals. Training emphasizes: (1) the understanding of chemical factors which predict a compound's ability to alter the genetic makeup of man, (2) the development of reliable test systems with unequivocal quantitative relevance to man for detection and quantification of mutations in germinal cells, and (3) the elucidation of molecular and cellular mechanisms in mutagenesis.

This is the smallest area of NIEHS training support and is comprised of one institutional award (University of California, Berkeley - Dr. Stuart Linn) and two postdoctoral fellowships.

New York University

Research in Chemical Carcinogenesis. There is a consensus, based on experimental and epidemiological evidence, that the induction of human cancer involves a multi-step process and extends over a period of years. The majority of human cancers are thought to be caused by agents such as chemicals, radiation and viruses, although genetic susceptibility may be variable. A major objective of research in this Center is cancer prevention. Research involves an attempt to identify carcinogens and anticarcinogens in both the occupational and general environments, assess the implications to humans facing possible exposure, and determine the mechanisms of cancer induction at the molecular, cellular, and tissue levels.

The hypothesis that nutrition has a large effect on cancer occurrence in populations throughout the world is being explored. Vegetarians and populations largely consuming vegetable proteins have strikingly low breast and colon cancer rates. Preventive agents in the vegetarian diet may be responsible for these differences. The occurrence of one type of tumor inhibitor present in human food, the protease inhibitors, is being studied. It has been found to occur in canned legumes and tofu. One inhibitor from tofu, the Bowman-Birk inhibitor, has been purified. This inhibitor is heat stable and is not destroyed by pepsin. Thus, these smaller inhibitors have the stability to survive cooking or roasting procedures used in food preparation, are not destroyed by gastric digestion and are effective in blocking an essential action of tumor promoters.

Studies have been carried out on in vitro cocarcinogenesis in order to gain a greater understanding of the mechanism of action of cocarcinogens. Dose response, cytotoxicity and transformation studies were carried out using an indirect-acting carcinogen benzo(a)pyrene [B(a)P], a direct-acting alkylating carcinogen β-propiolactone (BPL) and a mouse skin cocarcinogen, catechol. The rate of transformation was much higher in cell groups treated with B(a)P + catechol and BPL + catechol than in groups treated with either B(a)P or BPL alone. Catechol alone did not induce transformation. The rate of transformation was linearly proportional to the concentration of B(a)P (up to 0.02 μg/ml) and BPL (up to 2 μg/ml added). The transformed foci were grown successfully in soft agar, a property which best correlates with tumorigenicity in host animals.

In order to assess the role of DNA repair in chromate mutagenesis, a variety of bacterial strains defective in one or more DNA repair pathways have been compared. Results show that mutagenesis by chromate is unaffected by the uvrA-dependent excision repair pathway and is SOS-independent. This supports the view that chromate caused replication errors, perhaps by causing increased infidelity of the DNA polymerase.

Arsenite, a human carcinogen, acts as a comutagen with UV light in E. coli. When a variety of DNA repair-deficient strains were tested in a comutagenesis assay, only strains wild-type for DNA repair were positive, suggesting that arsenite acts as a comutagen by inhibiting the excision repair of pyrimidine dimers. However, DNA polymerase I is not inhibited by arsenite.

Investigations are continuing to determine whether UV-mutagenesis in mammalian cells occurs via errors in DNA replication or whether an inducible, error prone system, analogous to the SOS system in bacteria, is required. It was found that

the transcription inhibitor DRB (5,6-dichlor-1-β-D-ribofuranosyl-benzimidazole), when given to UV-irradiated Chinese hamster V79 cells, caused an enhancement of UV-mutagenesis. In contrast, treatment with the translation-inhibitors cycloheximide or puromycin caused decreased UV-mutagenesis. Thus, mutation frequency appears to correlate with the rate of post-irradiation DNA synthesis, suggesting a misreplication mechanism.

It has been shown that as rats become older, a given single dose of radiation becomes progressively less oncogenic. The yield of tumors for the same follow-up time becomes about 1/20 for 200d old rats in comparison to newborns. Follow-up studies have shown that the rate of repair of radiation induced single strand breaks declines progressively with age as does the rate of wound healing. The yield of epidermal DNA breaks was not age-dependent. The results are consistent with repair playing a major role in radiation carcinogenicity.

Research in Radiation Carcinogenesis and Dosimetry. Studies in progress in this area include development of instrumentation and calibration techniques to accurately measure low energy photon emitters in vivo in humans and experimental animals. Instrumentation is described to determine ^{222}Rn continuously at environmental levels in order to measure annual human exposure and develop a predictive model for environmental ^{222}Rn concentrations. Detailed human tracheobronchial dosimetry is being investigated in order to obtain estimates of the absorbed alpha dose from short-lived ^{222}Rn daughters and long-lived alpha emitters present in the atmosphere.

Research in Toxicology. Recent studies have dealt with the effect, in Wistar rats, of lower steady state levels of sulfite oxidase for longer periods of time than used in initial studies. The results showed that there was no increase in the tumor incidence above that observed in the previous studies and, thus, no evidence for a conventional treatment-response relationship. Experiments with C57Bl/6 mice similar to the rat studies failed to produce any mammary tumors in young females.

Preliminary experiments to develop a noninvasive method for the estimation of sulfite oxidase activity in intact animals, utilizing sulfite oxidase-deficient rats, have shown considerable promise. There is an inverse correlation between sulfite oxidase activity in rats and the excretion of urinary thiosulfate following loading with cysteine by gastric intubation. This correlation occurs over the approximate range of sulfite oxidase activity believed to be present in the human population. The objective of current experiements is to demonstrate the feasibility of predicting sulfite oxidase activity in rats from urinary excretion of thiosulfate following a loading dose of cysteine. The ultimate objective is to develop a similar test for humans.

Mice, pigeons and monkeys are being used as models to study neurotoxicity induced by the inhalation of organic solvents. Work thus far has involved the inhalation of benzene, with the determination of concentration-effect as well as time-effect relationships. Benzene, in concentrations ranging from 100 ppm to 1,000 ppm, alters consumption of milk by mice. These and other behavioral changes are being compared with the hematotoxic effects of benzene inhalation in the mouse. The results with benzene are being compared with the effects of toluene.

The pharmacology and toxicology of short-term memory function in both pigeons and monkeys is being studied. The delayed matching procedure used with these animals

provide an excellent model of memory function. Memory was impaired by acute administration of scopolamine, a cholinergic blocking agent previously shown to interfere with a variety of intellectual functions. In contrast, the stimulant drug amphetamine had no demonstrable effects on improving memory. A new peptide vasopressin analog (DDAVP) has been reported to improve memory function in humans, but DDAVP did not improve short-term memory in pigeons.

A study was completed of the effects of submicrometer sulfuric acid (H_2SO_4) aerosol on human pulmonary physiology. Eighteen healthy and ten asthmatic subjects inhaled H_2SO_4 via nasal mask for 1 hour at concentrations of 0, 100, 300, and 1000 $\mu g/m^3$ in a random sequence. The aerosol size (0.5 μm) and lower exposure level (100 $\mu g/m^3$) were selected to simulate peak ambient conditions, while the upper exposure concentration (1000 $\mu g/m^3$) is the current 8 h/d threshold limit for chronic occupational exposure.

Bronchial mucociliary clearance was markedly altered in all but one of the healthy subjects following one or more of the H_2SO_4 inhalations. The mucus was tagged by having each subject inhale either a 7.6 or 4.2 μm radiolabeled Fe_2O_3 aerosol. Exposure to 1000 $\mu g/m^3$ H_2SO_4 produced transient delays in clearance, which lasted longer in the group of eight inhaling the smaller 4.2 μm Fe_2O_3 aerosol than in the group of ten inhaling 7.6 μm Fe_2O_3. At 100 $\mu g/m^3$, the groups differed to a greater extent, with clearance of the more centrally deposited larger aerosol being stimulated and that of the distally deposited smaller aerosol being transiently inhibited. Tracheal clearance remained unchanged in both groups. These findings, along with calculations of the various aerosols' deposition patterns, indicate that H_2SO_4 exerted a greater effect in the distal airways than in the proximal airways. There were no statistically significant effects of the H_2SO_4 exposures on clearance for the group of asthmatics, possibly because of the much larger day-to-day variability in clearance rates in such individuals in comparison to nonsmoking, healthy subjects. On the other hand, slight changes in ventilatory mechanics were noted in the asthmatic subjects following the 1000 $\mu g/m^3$ H_2SO_4 exposure, an effect not observed in the healthy subjects.

Research in Respiratory Disease and Aerosol Physiology. The investigations currently underway are aimed at examining the initial deposition and subsequent translocation and clearance of particles from the respiratory tract. Both model test systems and *in vivo* test systems are being used.

An investigation is continuing on the effects of acute and chronic exposures to H_2SO_4 mist and acute exposures to an Fe (III)-S(IV) aerosol upon particle deposition, mucociliary clearance, and for the acute exposures, airway resistance. The rabbit is the animal model.

Exposures of animals via oral breathing to $\sim$ 250 $\mu g/m^3$ H_2SO_4 for five d/wk for four weeks resulted in a speeding-up of mucociliary clearance. Airway resistance was found to increase following one-hr exposures to H_2SO_4 at 2 mg/m^3, but no change was found following either a 1 or a 2 hr exposure at 1 mg/m^3.

Research in Epidemiology, Biostatistics and Biomathematics. Ongoing research activities in biomathematics, biostatistics and epidemiology pursue the following approaches: 1) theoretical formulation of mathematical models for extrapolation to low dose levels and for evaluating the temporal distribution of cancer and other diseases following chronic exposure to carcinogens and other toxic agents; 2) development of effective methods for the design of experiments and analysis of

data; and 3) epidemiological studies of cancer in human populations, to identify carcinogenic risk factors and develop means for cancer prevention. Moreover, through an Interlaboratory Epidemiology Program, multidisciplinary interactions between epidemiologists and their clinical and laboratory colleagues have been fostered, so that clinical and laboratory methods may be combined optimally with epidemiological approaches.

About 2,700 children given X-ray therapy for thymic enlargement and their 5,000 siblings have been followed for X-ray effects. Analysis of data from a new survey of these groups has just begun. The thyroid doses ranged from five to over 1000 rads. Preliminary results indicate a strong dose-response relationship for both thyroid adenomas and thyroid cancers, with no indication of a dose-squared component for either one and with no evidence for a sparing effect due to dose fractionation. Analyses of host susceptibility factors showed that females and Jewish persons are at higher risk for radiation-induced thyroid cancer, but there was no interaction between sex and ethnicity.

A cohort of about 600 women given radiation therapy for acute postpartum mastitis between 1940 and 1955 are also being followed. A total of about 1000 women, consisting of female siblings and women treated without radiation for the condition, serve as the controls. The irradiated women showed a two-fold increase in breast cancer overall. The dose-response relationship was approximately linear up to a high dose level.

Data acquisition has been completed in a study of the effects of childhood lead poisoning on later neurospychological performance. Forty-eight sets of twins or triplets who were discordant in childhood lead poisoning, according to records of the New York City Department of Health, have been tested and given physical examinations. Acquisition of detailed medical histories has permitted sub-classification of the subject population according to degrees of historical lead exposure. A significant deficit in intelligence as measured by the Wechsler Intelligence Scale for Children (Revised) has been found between lead poisoned twins when one achieved blood levels of 60 μg/dl or more and the control twin siblings blood lead level never exceeded 20 μg/dl. There were no significant differences in intelligence scores in groups of twins with lesser differences in lead exposure. Similar patterns have been found in the scores of standardized reading tests obtained from the subjects' school records. Analysis of other data obtained in the study is currently underway.

The relationship of aircraft noise levels to reading deficits and to sensorineural hearing loss among elementary school children in the public schools of Brooklyn and Queens is being studied. The aircraft noise levels were based on the Noise Exposure Forecast contours for the New York City airports, which were found to have high correlations with other independent noise level measurements. The percentage of students reading one or more years below grade level in each school was regressed on the estimated aircraft noise levels, controlling for various sociodemographic and teacher-characteristics variables. The results indicated an increment of about 5% of students reading below grade level could be associated with the increased noise levels in the noisiest schools. The risk of sensorineural hearing loss was about doubled in the noisiest areas, but due to the relatively small numbers the excess was not statistical significant.

Research in Environmental Pollution and Ecology. Environmental Health includes the impact of air and water quality on human populations exposed either to

contaminants or toxins in these media, or to food organisms which come from them. In this section, reports document progress in a variety of disciplines focussed on the interaction of contaminants with various media, and the direct and indirect impact of environmental contamination on humans. Since the fate and distribution of contaminants and toxins in air and water is intimately related, emphasis is placed in this section on transport phenomena, whereby selected compounds can be traced in their progress from the environment to man, whether the route involves direct effects due to inhalation, or requires biological intermediaries consisting of terrestrial estuarine and marine food chains.

A study is being carried out on the rates of PCB transport in model food chains to determine the extent to which PCBs in estuarine and coastal waters and sediments may accumulate in organisms used as human food. Two model food chains have been used. One, simulating an estuarine system, includes sediments, Neanthes succinea, and the hogchoker, Trinectes maculatus. The marine model includes sediments, Nereis diversicolor, and the winter flounder, Pseudopleuronectes americana. Some experiements have been carried out using striped bass (Morone saxatilis) and, as a food organism, the amphipod, Gammarus tigrinus. PCB food chain kinetics are being determined using ^{14}C-labelled Aroclor 1254.

The data are being used in pharmacokinetic models useful in estimating critical aspects of tissue partitioning, tissue clearance, and the potential for fish to depurate body burdens. Also critical to food web models are studies aimed at estimating the relative behavior of PCB isomers, or isomer classes in marine food organisms. Hogchokers apparently eliminate lower chlorinated isomer groups more rapidly, thereby accumulating body burdens of PCBs which superficially resemble Aroclor 1254 in isomer distribution. However, the available data suggest that individual tissues have widely differing capacities to selectively retain, or eliminate, PCB isomer classes.

Mount Sinai School of Medicine

Long-Term, Low-Level Exposure to Environmental Agents. The health status of a group of workers very heavily exposed to titanium (the work force at the oldest titanium-producing plant in the United States, in St. Louis) has been examined. All data have been collected and the statistical analyses now continue. Clinical studies show that no unusual abnormalities were present.

In studies of lead-exposed workers, neurobehavioral changes were investigated. These included measurement of nerve conduction velocity. Slowing of the median and sural sensory nerve conduction velocity was found. Median motor nerve involvement was detected with increase in duration of exposure, and sensory nerve effects seem to precede the development of motor nerve changes. There were suggestions that the sural nerve may be more susceptible to the early toxic effects of lead than the median or peroneal motor, and the median sensory. Another neurological index of dysfunction was saccadic eye movements. The studies indicated that the ability to execute eye movements smoothly from one point of visual fixation to the next in sequence was disrupted by exposure to inorganic lead. Further, the velocity of such movement was affected. These studies indicate that quantitation of ocular motor performance may provide a sensitive early indicator of lead effects on the central nervous system control of saccadic eye movements.

Neurobehavioral effects of lead in automobile workers were also investigated, using a battery of performance tests previously employed in studies of lead-exposed groups. Investigation of the performance of the automobile assembly plant workers showed trends of deteriorating performance after three to five years of exposure. When these results were compared with those of other occupational groups with varying levels of lead exposure, it was clear that lead-related central nervous system dysfunction in adults can be demonstrated by psychometric methods. Effects appear to be dose-related. Of further interest was the fact that zinc protoporphyrin levels were a consistently better indicator of lead effects than blood lead levels.

The effect of cessation of cigarette smoking among asbestos-exposed workers has been studied. New data have been obtained concerning effect on life expectancy of the various combinations of asbestos and cigarette smoking. The experiences of the American Cancer Society's blue collar workers were used, taking into account age, onset of employment, amount of cigarette smoking, current cigarette smoking habits, and duration of smoking. A significant reduction in life expectancy was found for insulation workers overall, but this was very much related to whether or not there was a history of cigarette smoking and, to a lesser extent, to age when smoking was begun. In the short run, the insulators did as well as the controls. Only those who started work late in life showed any indication of adverse effects prior to twenty years. Thereafter, the picture became increasingly bleak for the cigarette smokers relative to the controls. Only 34 percent of the workers who started work at about age 20 might expect to live 50 years, compared to 61% of the control cigarette smokers and 75% of nonsmokers among blue collar workers in general. On the other hand, insulators who were nonsmokers showed no reduction in survival relative to nonsmoking controls for the first thirty years, although a reduction of appreciable magnitude occurred in later decades. For cigarette smoking insulation workers, there was a loss of life expectancy compared to cigarette smoking controls of 11% and 17% compared to nonsmoking controls. When insulators stopped smoking, there was an increase in life expectancy but for those under 25 at the start, there was still a large deficit in life expectancy compared to controls.

Using experiences reported for several occupational cancers, the influence of both dose and time from onset, for environmental human cancer has been analyzed. The first analyses were for studies concerning radiation and available information concerning other occupational carcinogens, as betanaphthylamine and benzidine, lung and sinus cancer among nickel workers and lung cancer in uranium mining. It was found that most agents demonstrate a linear dose-response relationship with no evidence of a threshold. Any added exposure carries an added risk. The effect of exposure to some important carcinogens is to multiply a pre-existing risk at a given site by a factor relating to dose and to continue to multiply the risk in the absence of exposure for several decades. As this "background" risk is low in younger years, few cancers may be seen. However, when the "background" rate rises to a significant level at older ages, the effect can be dramatic. It is clear that carcinogenesis is an extremely complex process for which factors other than exposure are of considerable importance (repair, immune competence, etc.). These may help determine the observed age-dependence of cancer in humans. It is suggested also that much human cancer is multifactorial in origin.

Environmental Cancer. A study has been completed on pulmonary neoplasms induced by vinyl chloride. The report is currently in press and demonstrates the very considerable occurrence of such neoplasms.

The ultrastructural changes seen in the mouse lung tissue is similar to those of hepatic cells of animals exposed to vinyl chloride. The fact that vinyl chloride has a tendency to be soluble in lipid substances suggested that the lipid-rich pulmonary cells (Type II and alveolar-septal cells) might bind to vinyl chloride. If this assumption is correct, at least some of the ultrastructural alterations might have resulted from this mechanism.

An important asbestos-associated problem has been identified in the chemical and oil refining industries. Maintenance workers (in many plants, this group includes more than half of all employees) have been found to have radiological stigmata of asbestosis. Initial studies showed a significant prevalence of chest X-ray abnormalities in both chemical plant maintenance workers and oil refinery workers, with prevalence virtually the same whether the worker had previous asbestos exposure or not. Pleural abnormalities were more prevalent than parenchymal. There were comparatively few subjective symptoms or pulmonary function abnormalities. While the risk of developing severe disabling pulmonary fibrosis seems unlikely, the risk of lung cancer and/or mesothelioma or other neoplasms would appear to be higher than normal.

Potentially important laboratory observations have been made among patients with mesothelioma and asbestos-exposed workers without this neoplasm. In more than 80 patients with mesothelioma, immunosuppression was found, with decreases in numbers of T-cells and some decrease in B-cells, increase in "null" cells, and poor response to phytohemoglutin, pokeweed antigen and in mixed lymphocyte cultures. Also, there was significantly altered response to a number of recall antigens. In several patients, however, there was no evidence of immunosuppression, with normal lymphocyte studies. These patients did well, one surviving for ten years and several others alive at four and five years from onset of symptoms. To obtain further information, lymphocyte studies were done in 143 consecutive asbestos insulation workers, each approximately 30 years from onset of exposure without evidence of cancer but with X-ray evidence of asbestosis. Approximately one-third showed lymphocyte changes consistent with immunosuppression and alteration in response to recall antigens was again found. Using absolute or relative measures of exposure for a number of asbestos-employed groups, it has been possible to calculate the asbestos-related cancer mortality, using data for asbestos insulation workers for estimating dose and time dependence of asbestos cancer for all other trades. In this way, asbestos-related risks are expressed relative to those of insulators. Estimates of such relative risk come from information on those mortality studies available, asbestos air concentrations, prevalence of abnormal X-rays and in long-term employees and the ratio of cases of mesothelioma found in national studies. The estimates indicated that from 1940-1979, approximately 13,200,000 individuals had significant potential asbestos exposure at work, and 9,200,000 are estimated to have been alive on January 1, 1980.

At the present time, approximately 8,500 asbestos-related excess cancer deaths are occurring annually. This will rise to about 10,000 annually by the year 1990. Thereafter, the mortality rate from past exposure will decrease, but still remain significant for another three decades.

These projections of cancer mortality for asbestos-exposed workers have been based on past experience to asbestos. (They do not include estimates of similar disease among family contacts, use of consumer asbestos products, neighborhood exposures, etc.).

The fibrous zeolite mineral erionite has been assumed to be the agent responsible for an unusually high incidence of pleural mesothelioma reported among inhabitants of three villages in south central Turkey. Zeolites are extraordinarily abundant and widespread in the volcanogenic sedimentary rocks in this area. They have also been reported in 40 additional countries, including the United States, where most states west of the Rocky Mountains have zeolite minerals.

Environmental samples were collected in villages from which mesotheliomas were known to occur, as well as from those where mesotheliomas have not been reported, from settled house dust, interior white washes, settled dusts and soils from roads, schoolyards, cultivated fields, tuff from schools and house walls, and public drinking water. Lung tissue specimens were obtained from patients with mesothelioma who had lived in the villages of Karain, Tuzkoy and Sarihidir. The specimens were analyzed by polarized light microscopy, X-ray powder diffraction and by transmission electron microscopy.

The environmental samples showed many erionite fibers, fine and short, approaching the dimensions of some asbestos fibers. Seventy-five percent were less than 0.25 um in diameter and virtually all were less than 3 um in length. In addition, the samples contained several different kinds of fibrous minerals other than erionite. In Karain, for example, amphibole fibers and chrysotile were found. In one alteration zone, 30-40% by weight of the specimens were found to consist of tremolite and chrysotile.

Lung tissues of mesothelioma patients were found to contain both fibrous zeolites and asbestos minerals. Although the first mesotheliomas were reported from Karain and Tuzkoy, pleural mesothelioma has recently been diagnosed in a third Cappadocian village, Sarrihidir. Samples of street dust collected in Sarrihidir contained as much as 5% chrysotile and tremolite, by weight, confirmed by transmission electron microscopy and microchemical analysis.

With the finding of mesotheliomas in Cappadocia, Turkey, and the simultaneous identification of fibrous zeolites in this area, it was considered useful to investigate the carcinogenic and fibrogenic potential of the minerals in an experimental model already shown to be effective in demonstrating experimental mesothelioma with asbestos. Two natural fiber zeolite minerals, erionite and mordernite, were injected intraperitoneally in Swiss mice. Untreated mice remained as controls and five mice treated with chrysotile were used as positive controls. Six of ten mice treated with erionite developed malignant peritoneal tumors between 8 and 22 months after a single administration and four of the six neoplasms were consistent with malignant mesothelioma. Two of the four chrysotile-treated controls also developed tumors. A fibrogenic effect was noted in both the erionite and mordernite-treated mice, the effect being more pronounced in the former. The findings suggested that the carcinogenic effects of fibrous erionite are similar to those of asbestos.

Halogenated Aromatic Hydrocarbons. Tissue PCB burdens of 326 workers at two factories manufacturing and repairing electrical capacitors have been measured. Current exposure was mainly to di- and tri-chlorobiphenyls. Thus, exposure to the higher Arocolor was limited to those workers with more than five years of employment experience. From data given by the company and union and from inspection of the working areas, and from air sampling data, the jobs were categorized as "high", "medium" and "low" exposure.

Plasma and adipose concentrations were significantly higher for males than for females only among persons in the high exposure category. The highest levels of PCBs were observed among persons in the high exposure group, with people involved directly with the capacitor treatment having the highest exposure and concentrations. Altogether, there were 25 people with plasma PCB concentrations greater than 300 ppb. Of these, 20 worked in high exposure jobs and four had transferred from high exposure to low exposure jobs within the previous two years.

Two cross-sectional examinations have been made, one in 1976 and the second at the end of 1979, to determine if health effects have occurred. Few clinical abnormalities have so far been observed.

Adverse effects on spermatogenesis in man have been reported from exposure to halogenated hydrocarbons, such as dibromochloropropane, Kepone and chloroprene. Bulls removed from PBB-contaminated farms in Michigan had testicular atrophy and calves experimentally fed PBB also developed testicular atrophy. Thus, an analysis was made of semen samples from farmers residing on PBB farms. These individuals had bought food directly from such farms, or worked in the plant in which the chemical was made. A control group included male graduate students at a Michigan university who were considered to have consumed little or no PBB-contaminated food. All semen analyses were performed one hour (at most, three hours) after collection by the technician who regularly performed such routine hospital analyses at the Mount Sinai Hospital. Blood samples were also obtained from all men for measurement of serum PBB levels. Men from the PBB farm cohort who initially had "abnormal" results underwent a second semen analysis two to four months after the initial test. Altogether, semen analyses were performed in 104 men. No difference in distribution of sperm counts was found, nor in sperm motility or sperm morphology among the exposure groups, compared to the controls.

Massachusetts Institute of Technology

Basic Studies of Organic Particulates From Vaporizable Fuel Combustion. Major progress was made in the study of the formation of PAH and soot in the well-stirred reactor. New findings of special interest include:

a. For the two fuels studied, toluene and ethylene, production of PAH maximized at the sooting limit (the fuel/air mixture ratio where the transition from a non-sooting to a sooting flame occurs). This observation indicates that the growth of soot depletes the building blocks for PAH formation. This observation is of practical interest since measures taken to reduce soot could increase PAH formation. The same effect was observed in the domestic oil burner studies carried out by this program.

b. Increasing flame temperature greatly reduced PAH formation. For example, in a toluene flame, increasing temperature from 1580°C to 1670°C reduced the maximum pyrene production from 30µg/g toluene to 2ug/g toluene.

c. Comparison of flat flame composition with composition from the well-stirred reactor showed a strong similarity; however, the very high PAH concentration found in the flame zone of the flat flame, which

does not exist in the well-stirred reactor, indicates that quenching of laminar flamelets by cold combustion chamber walls or by mixing with cold gas can be a major source of emissions which would be absent in an intensely mixed system.

Basic Studies of Organic Particulates From Coal and Heavy Liquid Fuel Combustion. The Coal experiments were carried out in a drop tube furnace where coal particles can be subjected to a controlled temperature, time and atmospheric history.

Soot yields were found to increase with increasing residence time in the furnace, finally approaching an asymptotic value. The bituminous coal gave much higher yields of soot than the lignite as would be expected from the larger number of aromatic rings in the basic building blocks and the lower oxygen content of the bituminous coal. Anthracite coal, however, has a low volatile content and gave relatively low soot formation.

A finding of interest is a very sharp transition from low molecular weight PAH compounds (3 rings or less) collected in the vapor phase to compounds of 3 to 7 rings found on the soot. These results confirm expectation that a sequential condensation of PAH will occur on soot particles with higher molecular weight compounds forming an inner core coated by the lighter compounds. This stratification of composition of PAH on soot may have ramifications for health effects to the extent that exposure in the lungs may be limited to the surface layers on the soot. The data currently being interpreted will provide insights on the relative amounts of soot and PAH, their dependence on coal type and furnace temperature, and their interaction in the colder parts of a furnace.

Combustion Studies of Particulates.

a. Turbulent diffusion flames: Both axial and radial profiles of soot and PAH species concentrations were obtained. Measurements of the decay of pyrene, fluoranthene, 2-methylnaphthalene, and phenanthrene indicate that the oxidation of these species occurs at a much lower rate than their destruction by pyrolysis in the region closest to the burner nozzle. Decrease in bioactivity is observed for samples taken at increasing distances from the nozzle; this implies that even though the fuel contains mutagenic PAH it will probably be feasible to achieve combustion products with low levels of active PAH species concentrations. In both staged and unstaged flames the azial profiles of PAH concentration show rapid decrease of PAH as soot is being formed. At large axial distances where most of the oxidant has been entrained into the jet, it was observed that the soot concentration approaches a low symptotic level. This level is believed to be determined by freezing of the soot oxidation process due to quenching.

b. Fluidized bed combustion: A large fraction of this year's effort was devoted to developing sampling and analytical techniques for highly particulate laden gasses encountered. Similar quantities and distribution of PAH were present in the incomplete combustion products withdrawn from the freeboard during fluidized combustion of both coal and oil shale. Nitrogen and sulfur-containing PAH were identified in all samples. The mass fraction of nitrogen in the PAH was approximately proportional to the dry, mineral-free nitrogen content of the fuel. No correlation of the amount of sulfur-containing polycyclics

with the sulfur content of the fuel was observed. Among the PAH identified were some which have been reported to induce forward mutation in Salmonella typhimurium. It will be important to determine the range of temperature, excess oxygen and residence time which is required for the destruction of these species before quenching of the combustion products at the outlet from the combustor.

Analytical Chemistry for Combustion Diagnostics: This year emphasis was placed on improving present analytical methods and widening the scope of analytical methodology to include compounds of widely ranging polarity and volatility. The method improvement goal led to a fractionation scheme based on alumina column chromatography. It was implemented to provide class separation of combustion effluent extracts prior to analysis by capillary gas chromatographic-mass spectrometry.

The fractionation scheme was used in the characterization of the chemical composition of domestic fuel oil.

A high performance liquid chromatography system has been installed to provide new information on samples which are not amenable to GC-MS analysis and to provide complementary data for those which are. The system is based on a ternary liquid chromatograph coupled to a rapid-scanning spectrophotometer.

Single Cell Assays for Gene Locus Mutation: A mutation assay has been developed with human lymphoblasts competent for oxidative xenobiotic metabolism. This novel human cell line was capable of activating a variety of xenobiotics to mutagens. Among the compounds found to be active mutagens were the soot components 1-methyl phenanthrene, benzo(a)pyrene and cyclopenta(c,d)pyrene. Fluoranthene and perylene were not active mutagens in this system. The lack of fluoranthene-induced mutagenesis may be due to low levels of epoxide hydrolase in this cell line. Studies with fluoranthene, a potent human and bacterial mutagen found in high concentrations in the methylene chloride extract of diesel soot exhaust, have been extended to variant human lymphoblast which are particularly sensitive to mutations by fluoranthene.

A new "micro" forward mutation assay in S. typhimurium has been developed. This assay has been applied to the direct measurement of mutagenecity on HPLC fractions. This technique was found to be a useful tool for the identification of toxic and mutagenic components in complex mixtures. The technique was applied to the analysis of diesel exhaust, fluoranthene metabolites and residential oil burner effluent. The analysis showed that the mutagenicity of toluene combustion products in bacteria can be accounted for by the presence of fluoranthene and cyclopenta(c,d)pyrene.

Finally, studies have begun to identify and characterize the toxicity and mutagenicity of partially hydrogenated aromatic compounds. The partially hydrogenated products tested did not induce significant mutation in bacteria in the presence or absence of PMS. The hydrogenated products did demonstrate increases in acute toxicity, particularly in the case of dihydro and octahydrophenanthrene.

Modification of Macromolecules In Vivo and In Vitro By Products of Fossil Fuel Combustion. Fluoranthene, a major polycyclic aromatic hydrocarbon product of

fossil fuel combustion, is metabolized by microsomal enzymes to mutagenic species which bind to DNA. The major site of covalent modification of DNA by fluoranthene metabolites occurs putatively at guanine.

Metabolism studies indicated that the ultimate mutagenic forms of fluoranthene which bind to DNA are vicinal dio-epoxides rather than fluoranthene arene oxides. The initial formation of the 2,3-dihydroxy-2,3-dihydrofluoranthene which is then oxidized to the 2,3-dihydroxy-1,10b-epoxy-1,2,3-trihydrofluoranthene is proposed as a major metabolic pathway responsible for the mutagenic potency of fluoranthene.

The majority of the fluoranthene metabolites have been identified. The spectrum of the identified metabolites demonstrates that fluoranthene is extensively metabolized throughout the molecule by both purified Aroclor-induced rat-liver microsomes and postmitochrondrial supernatant. Metabolites were identified by: (1) HPLC co-chromatography with synthetic standards, (2) comparative UV-visible spectroscopy and/or(3) mass spectroscopy. The use of radiolabeled 3-(^{3}H)-fluoranthene facilitated the quantification of the metabolites.

Metabolites were isolated from the reverse-phase HPLC metabolite profile, subjected to further purification by HPLC, and their structures determined. The metabolites which can account for up to 80% of the total metabolism were determined to be; 2,3-dihydroxy-2,3-dihydrofluoranthene, 3-hydroxyfluoranthene, 2,3-fluoranthenequinone, 1-hydroxyfluoranthene, 8-hydroxyfluoranthene and the 1,10b-dihydroxyfluoranthene. In separate experiments, mutagenicity data from the S. typhimurium forward mutation assay indicate that 2,3-dihydroxy-2,3-dihydrofluoranthene, which is quantitatively the major metabolite, also accounts for up to 85% of the observed mutation. When metabolism incubations and mutation assays were treated with 100 μm TCPO, both the formation of the 2,3-dihydroxy-2,3-dihydrofluoranthene and the observed mutant fraction were decreased to a similar extent. TCPO inhibits the epoxide hydrolase catalyzed conversion of arene oxides to trans-dihydrodiols. These results suggest that fluoranthene arene oxides do not contribute significantly to the mutational potential of fluoranthene and demonstrates that a major proximate mutagenic form of fluoranthene is the 2,3-dihydroxy-2,3-dihydrofluoranthene.

The binding of fluoranthene metabolites to DNA has been optimized to approximately one fluoranthene moiety per 8000 nucleotides. This binding level has allowed for the isolation of ug amounts of a major fluoranthene-DNA adduct. The purified adduct was treated with $HClO_4$ which released a stoichiometric amount of guanine. This would be analogous to what has been observed for the carcinogenic PAH, benzo(a)pyrene. It is implied from the metabolism and mutation data that the fluoranthene moiety bound to DNA is derived from the 2,3-dihydroxy-1,10b-epoxy-1,2,3-trihydrofluoranthene.

Oregon State University

Research was pursued on a variety of chemicals to which humans may be exposed. Among these are the polychlorinated biphenyls, 2,4,5-T, pentachlorophenol, methylmercury, halogenated hydrocarbons, aflatoxins and pyrrolizidine alkaloids. Studies included determination of the biological effects on whole organisms, tissues and cells, biochemical mechanisms of action, dynamics of the chemicals in organisms and the environment, and modeling of the dynamics of chemicals.

Parameters of toxicity and immunologic function were examined in adult male and female mice exposed to 2,4,5-T. No overt toxicity was seen at levels as high as 250 ppm. Body weights and selected organ weights (liver, spleen, thymus) were unaltered by 2,4,5-T exposure.

T-cell blastogenesis (Con A, PHA) was enhanced in male mice exposed to 2,4,5-T for six weeks, whereas the B-cell response (LPS) was enhanced in female mice. No changes in mitogenicity were apparent after 12 weeks of exposure in either male or female mice.

From these preliminary results, chronic 2,4,5-T exposure does not appear to present an immediate concern from an immunotoxicity standpoint. The results also suggest that the level of TCDD (0.088 ppm) in the 2,4,5,-T was sufficiently below the level necessary to cause immunotoxicity. The 0.088 ppm TCDD level in our 2,4,5-T sample is near the 0.1 ppm level established as acceptable by the Environmental Protection Agency.

Wistar, Fischer, Long-Evans and Sprague-Dawley rats were compared to OSU Wistar strain rats in the model system and found to be less sensitive for reproduction and survival to weanling age parameters when exposed to methylmercury and nitrite. Progeny from all strains did develop neural tumors within three months. Addition of NO_2 in the diet instead of in the drinking water was tested as part of the rat strain comparisons and proved to be less toxic than in the water. The Charles River Wistar strain was selected for future experiments and used for initiation of a MeHg dose-response experiment. The highest level of MeHg (15 ppm) and highest level of NO_2-EU (1.5 g NO_2/l water-4.8 g EU/kg diet) produced the most toxic effect on litter size, birth weight, percentage of stillborn progeny and survival to weanling age. However, the increase in toxic response was not consistently linear with increasing MeHg level. The effect of MEHg on survival to weanling age was nearly linear with increasing dose of MeHg. Measurement of the effect of MeHg dose on incidence and latency of neural tumor development is in progress.

The effect of lipoproteins LDL, HDL and VLDL on lipidosis induced by hexachlorobiphenyl (HCB) in skin fibroblasts was determined. LDL and VLDL induce lipidosis when the cells are incubated with these lipoproteins in the absence of HCB, but HDL does not. Treatment of both normal skin fibroblasts and hypercholesterolemic type II skin fibroblasts with HCB induces lipidosis. Removal of the HCB and subsequent treatment with HDL clearly reverses the lipidosis; VLDL does not. LDL inconsistently only partially reverses the lipidosis. These results suggest that lipoproteins are involved in the transport to and removal of HCB from fibroblasts. The nature of the binding of HCB by serum albumin (BSA) and LDL was determined by analysis of the data using several standard plots. Hyperbolic binding curves were observed with albumin and LDL, indicating a non-cooperative binding. A single binding site was observed with BSA (n = 1) and a large number of sites with LDL (n = 30). The sites are noninteractive.

Male rats were fed a diet containing 80% lyophilized milk from female goats that were being fed a diet of 25% tansy ragwort (Senecio jacobaea). While no excessive mortalities were experienced, after one year the rats showed moderate liver pathology characteristic of PA poisoning. In another study, Wistar rats fed for one week on a contaminated goat's milk diet showed decreases in hepatic cytochrome P-450 levels and in the activities of related monooxygenases and increased epoxide hydrolase and glutathione-S-transferase activities, responses identical to those produced by the tansy ragwort PAs.

Experiments in rats and mice showed that the antioxidants butylated hydrooxy-anisole (BHA) and ethoxyquin protected against the toxicity of the mixed tansy ragwort PAs and the PA monocrotaline. Cysteine offered little protection against the PAs while diethylmaleate increased toxicity. In other experiments, dietary copper was found to accelerate intoxication by the PAs while addition of zinc to the diet markedly decreased the adverse effect of the PAs. Suggestive evidence was obtained that the latter protective response was due to both the reduced formation of the active pyrrole metabolites of the PAs coupled with an accelerated degradation of the PA pyrroles, perhaps via binding to the increased cellular levels of metallothionein.

Edible plants can accumulate high concentrations of metals, including Cd, from the soil, after the soil has been treated with sewage sludge as a conditioner. There is, thus, the potential for Cd contamination of human food supplies. Since Cd is a toxic metal, feeding studies were conducted in rats to help assess the potential hazard to man through the consumption of grains and vegetables which contain excessive concentrations of cadmium.

Female rats were fed semisynthetic diets containing 0.25 μg ^{109}Cd/g and 10% freeze dried spinach for various periods of up to 12 weeks. The rate of Cd accumulation in livers and kidneys on all diets was linear up to the eighth week of feeding, and then decreased markedly. Uptake of inorganic ^{109}Cd was increased by more than twofold when spinach was excluded from the diet. Uptake of plant-bound Cd was slightly greater than rate of Cd added to the diets in the inorganic form.

Research was conducted to examine pyrrolizidine alkaloid structure relative to the structural components necessary for inducing production of avian tumor virus synthesis by uninfected chick tissue culture cells. The endogenous tumor virus genes are a group of "silent genes" in avian cells that can be activated by certain chemicals. It was demonstrated that jacobine, a major alkaloid found in tansy ragwort, was capable of inducing virus synthesis in uninfected cells. Certain modified chemical derivatives of jacobine will induce virus synthesis, while other modifications of the structure of this chemical render it inactive. The importance of these findings are that chemical structure affecting the expression of cellular genes can be altered to change this biological activity. It may be possible to describe the types of reactive groups on a particular chemical structure that are responsible for causing change in the expression of the cellular DNA.

The effect of technical pentachlorophenol exposure on the humoral immune response has been examined. Exposure levels to PCP ranged from 50 to 500 ppm PCP in the diet for eight weeks prior to antigen sensitization. Cyclophosphamide-treated mice were included as a positive immunosuppressant control. The number of plaque-forming cells (PFC) in the spleen following sensitization with a T-dependent (SRBC) and a T-independent (DNP-Ficoll) antigen was examined. A highly, significant dose-dependent reduction in the number of PFC was observed in PCP-exposed animals after challenge with either SRBC or DNP-Ficoll. Both the primary and secondary anti-SRBC responses were significantly suppressed. In parallel with the reduced anti-DNP PFC response, suppressed circulating antibody titers were also observed in PCP-exposed mice. The level of suppression induced by 250 ppm PCP was equivalent to the suppression induced by a 27 mg/kg ip injection of cyclophosphamide. A direct effect of PCP on the B-cell is suggested by these findings.

The extent and nature of DNA phosphotriester formation and the biological consequences of 2-haloethylnitrosourea alkylation of DNA is being investigated. Particular importance is being given to the nature of interstrand crosslinking between DNA, DNA strand breakage and DNA and protein crosslinkage resulting from 2-haloethyl alkylation events during 2-haloethylnitrosourea degradation.

Plasmid pBR322 DNA is being isolated from E. coli RR1 after amplication. The method was modified to obtain a high yield (85%) of DNA in the supercoiled form whereas the amounts of relaxed and linear DNA are 10 and 5%, respectively. The supercoiled plasmid DNA migrates as a single band in a CsCl gradient containing ethidium bromide. All experiments involving ethidium bromide were performed under red light. Radio-labeled plasmid DNA was obtained by performing the amplification step in the presence of (methyl-^{3}H)thymidine. Quantification of the different forms of (^{3}H)DNA was done by agarose gel electrophoresis and liquid scintillation counting of the separated bands. Gel electrophoresis was performed in a horizontal slab gel system.

Plasmid DNA alkylation by either BCNU or chlorozotocin produced extensive damage including elimination of both supercoiled and relaxed forms. Kinetic studies have been performed and a careful evaluation of the nature of the linear form(s) generated during alkylation has been made. Plasmid DNA does not appear to possess any "hotspots" that are more sensitive to alkylation dl than any other regions of the DNA. Linear DNA was not formed by a double-stranded break in a specific position as shown by the formation of numerous fragments during digestion with BamHl, a restriction enzyme which cleaves this DNA in a single specific site. If a "hotspot" existed, the BamHl digestion would yield only two distinct fragments. Various procedures are being utilized to determine the nature of scission of the DNA strands during alkylation.

University of Rochester

In October 1980, it was discovered that several thousand infants, possibly as many as 12,000, had been exposed to phenylmercury in their diapers. Phenylmercury acetate had been used as a fungicide in the diaper wash by a number of diaper services in Buenos Aires, Argentina. The analytical findings indicated that several thousand infants had elevated mercury in urine with some values as high as 500 μgHg/l. Normal values are less than 20 μgHg/l.

A team from Rochester visited Buenos Aires in February 1981. Arrangements were made for the collection of samples of head hair to recapitulate past exposure and to start a long-term clinical followup of a representative sample of the exposed infants.

Studies on over 1000 people exposed to methylmercury in fish in Canada have indicated that the head hair is the sample of choice in recapitulating seasonal exposures. Once methylmercury has been incorporated into the hair at the time of formation of the hair sample, its concentration remains unchanged for many years.

An example of the misuse of a mercury fungicide on grain in Washington State has been found. The fungicide containing phenyl and methylmercury compounds was fed to poultry and the eggs consumed by a number of persons. Despite the much

higher concentration of phenylmercury in the treated grain, ten times more methylmercury than phenylmercury accumulated in the eggs. Blood levels of mercury in human subjects correlated with average daily consumption of the contaminated eggs but no cases of poisoning occurred.

Observations on humans exposed to methylmercury in the Iraq outbreak of 1971-72 revealed that the biological half-time of methylmercury in blood of lactating women was substantially lower than in non-pregnant, non-lactating women. This finding has been confirmed in animal tests. However, lactation has no effect on the same degree of degenerative damage in the granular layers of the cerebellum and in dorsal root ganglia as non-lactating females. However, lactation appeared to give some protection in terms of clinical signs such as weight loss and coordination disorders.

Investigations are continuing on factors which influence absorption, distribution, metabolism and elimination of mercury after exposure to methylmercury. Recent studies have shown that prior to weaning, suckling mice recycle mercury compounds between body proper and gastrointestinal tract lumen in a generally similar way to that observed in adult mice, but that the developing mice excrete very little mercury prior to weaning. We are currently examining possible effects of changes in gut microflora and demethylation on rates of mercury excretion in developing and adult mice. Further studies of renal mercury sexual dimorphism have documented apparent genetic (inbred mouse strain) differences in mercury excretion and organ distribution, with large male > female differences in mercury bound in kidneys of adult animals. These studies should aid in clarifying mechanisms of mercury excretion and possibly in design of treatment methods for accelerating excretion of mercury compounds in exposed individuals.

Possible effects of mercury compounds on reproduction are also being examined. Results to date have shown that body weights are reduced at weaning in a dose-related manner in offspring exposed to methylmercury as 12 1/2 day and 15 1/2 day fetuses. This dose-related weight reduction continues through eight months of age. A dose-related reduction of locomotor activity at eight months of age has also been observed. Analyses of reproductive performance of these mice whose germ cells, gonads, brains and reproductive organs prenatally exposed to a range of doses of methylmercury are continuing.

Tissue homogenates with Hg-cysteine and EtOH show an EtOH-dependent release of Hg vapor (mercury volatilization activity or MVA). Soluble fractions of liver homogenates were found to possess the greatest MVA activity. Both *in vivo* and *in vitro* evidence is presented that Hg^0-rich conditions in tissues favor Hg release into the vapor phase and that this may play an important role in the distribution and elimination of all forms of administered mercury.

Further studies on the induction of metallothionein (MT) have utilized the isolated, perfused rat kidney. Baseline information on substrate-limited and normal substrate conditions have been acquired along with the establishment of appropriate analytical methodology.

Normal male and female and orchidectomized male mice have been exposed to mercury vapor and $HgCl_2$. It appears that the sexual dimorphism observed may be due to differential uptake of mercury after oxidation of Hg^0; this leads to the lower detoxification rates found in the male kidney. Since orchidectomized male mice resemble female mice in this respect, the metabolism of mercury vapor in males appears to include regulation by sex hormones.

Cadmium distribution and MT binding have been further investigated in rats. The liver levels, biliary form, and retention times of hepatic MT-Cd association have been determined.

A single antibody radioimmune assay was developed and applied to rat plasma and urine. Urines of men and women exposed to cadmium have been assayed for MT, beta-2 microglobulin and Cd levels. Relationships of these parameters with exposure conditions and renal dysfunction are being investigated.

Human studies of air pollutants, e.g., sulfates, inorganic sulfates have continued to emphasize the comparative responsiveness of normals and selected groups of susceptible persons, e.g., asthmatics, in manifesting physiological airway changes. Dose-response information at three acute exposure levels has been acquired during oral breathing.

Chamber studies (nasal breathing) with exercise protocols have been initiated at 100 $\mu g\ m^{-3}$ levels for four hours. Two subjects are currently exposed simultaneously under double blind conditions.

Changes in bronchial and alveolar permeability are being evaluated in dogs as a potentially useful assessment method for investigating the effects of air pollutants in man. Techniques for collecting pulmonic lymph (dogs) for more protacted time periods have met with some success. Cadmium exposures of dogs have been undertaken to study lung MT induction, lymphatic transport and alveolar permeability changes. The cadmium studies have also been directed at developing a kinetic model of pulmonary lymphatic uptake and translocation.

Efforts are being made to delineate the molecular mechanisms of toxicity initiated by the interactions of xenobiotics with the cell's genetic regulatory systems. The actions of toxic agents that interact with the DNA directly or by interfering with receptor-mediated mechanisms are being studied. The receptor systems being investigated are those that control the cell's nuclear RNA synthetic capacity or the genetic expression of the cell.

The molecular properties of the estrogen receptor, the conformational changes induced by ligand binding leading to activation of the receptor and nuclear uptake of the receptor are being resolved. The interference by estrogenic antagonist and xenobiotics with these processes have been investigated during the past year as were the interactions of the tetrachlorodibenzodioxin (TCDD) with its specific receptor. Investigation of the role of TCDD metabolism in the toxicity of this substance has also been initiated.

University of Cincinnati

Progress was made in the studies of mechanisms of absorption of heavy metals from the intestine. Emphasis again focused on cadmium and zinc; additional experiments included nickel and copper. In spite of evidence suggesting that cadmium and zinc may compete for the same transport mechanism in the rat jejunum, evidence has now been obtained that the two systems do not react similarly to physiological changes in the animal. Thus, zinc-deficient rats are well known to be able to absorb zinc more efficiently than zinc-replete rats. Study of such animals with cadmium has now shown that the two groups absorb cadmium at the same rate. Clearly, the system responsible for zinc absorption reacts

differently to the physiological stimulus of zinc deficiency than does the system mediating cadmium absorption. Another difference between the two systems is that the second step in jejunal metal absorption (i.e. the transfer of metal from mucosa into blood) proceeds relatively much faster for the essential metals zinc and copper than for the toxic metal cadmium.

In the renal field, major attention has focused on definition of the mechanism whereby heavy metals inhibit amino acid reabsorption. It has been demonstrated that the site of inhibition lies at the luminal border of the tubular epithelial cells. Methods have been devised for the measurement of transport kinetics of amino acids from lumen to venous blood. It was necessary to measure tubular saturation of the carrier system and its affinity for substrate under conditions where metabolic changes would be minimized. Utilizing either bolus injections or a previously described special arterial infusion technique, it has proved possible to establish for at least one amino acid the uncompetitive nature of the inhibition. This implies a decrease in both the maximum rate of reabsorption and the affinity constant (K_M). Studies so far have utilized uranyl acetate to produce acute renal failure. The work is being extended to different amino acids and metals.

In a study of aluminum, the metal was administered to rats by mouth. Neuritic plaques and intraneuronal dengeneration of neurofilaments were observed in the brains of these animals. Changes in Cu and Zn levels in these areas again point to the possibility that Al toxicity, as well as that of other toxic metals, may be related to interactions with essential trace metals. This hypothesis has served as basis of much work on trace metals in the past.

The epidemiologic study of reproductive effects of women occupationally exposed to styrene continues. The study is being conducted through questionnaires and interviews.

In a group of workers exposed to toluene diamine and dinitrotoluene a significant depression of sperm count was observed in workers exposed to the two agents as compared to the unexposed control group from the same plant.

A population of workers exposed to a dry bleach product containing esperase enzyme was studied. Three of 12 workers were noted to have a positive RAST for esperase enzyme. In addition, post-work pulmonary function tests were significantly reduced in exposed workers.

A study of aflatoxin exposure associated with dust from contaminated corn continued during the year. The average concentration of aflatoxin in these dusts during the operation of a combine was 90 ppm. No clear relationship could be found between these levels and the concentration of the toxin in bulk corn. Additional sampling was conducted in the neighborhood of grain elevators and also at various locations about a combine harvesting corn. Possible hazards were found in southern states where high temperature and moisture favor formation of aflatoxin, but not in northern states.

An investigation of carcinogen metabolism involved the study of dibenzocarbazole, an N-heterocyclic aromatic compound. When this carcinogen was placed into the trachea of an isolated perfused lung, only approximately one-third of the compound originally instilled remained as such at the end of 120 min of perfusion. A large fraction of the original compound and of total metabolite could be recovered

from the tracheo-bronchial tree. This suggests that the pulmonary alveolar macrophage may deposit compounds in the bronchi, a conclusion consistent with the high incidence of respiratory tract tumors.

The mutagenic potential of coal hydrogenation products was investigated. The tests were performed with the Salmonella/microsome assay system. Mutagenicity of coal-related material was affected by a variety of factors including the ash content of the coal, and an increase in the amount of hydrogen in the reaction with consequent decrease in the yield of residual fuel oil.

In another study the effects of ethanol on vinyl chloride carcinogenesis were investigated. Ethanol was found to serve as co-carcinogen in vinyl chloride induction of tumors in the liver. Presumably ethanol acts by altering vinyl chloride metabolism, maybe by virtue of a common step in the oxidation of the toxic agents. The most likely candidates here are acetaldehyde and chloroacetaldehyde; the normal substrate, acetaldehyde, would be oxidized preferentially, resulting in an increased half-life on the chloro analog.

Organ cultures of the limb buds and intact embryos have been established which permit analysis of the mechanism of action of teratogens such as cytosine arabinoside, cyclophosphamide and cadmium. A variety of solvents, including four chlorinated hydrocarbon solvents and carbon disulfide, have been examined for reproductive effects with inhalation exposure of female rats. None have induced teratogenicity, while methylene chloride was shown to cause neurobehavioral deficits with maternal inhalation exposure to 4500 ppm. The effects of maternal inhalation exposure to vinyl chloride with and without 5% ethanol on teratogenicity have been examined with negative results, with the exception of an increase in skeletal variants in term fetuses. Under identical exposure conditions, however, vinyl chloride does induce transplacental carcinogenesis.

The target embryonic organs in the teratogenicity of the herbicide, Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) have been identified. The heart and diaphragm are malformed in such a way as to induce respiratory distress and death in newborns.

The transplacental carcinogen ENU (ethylnitrosourea) has been studied as a potential positive control for neurobehavioral studies. Transplacental exposure of fetuses leads to neurobehavioral deficits in the offspring several months prior to the appearance of tumors in the central and peripheral nervous system.

Fundamental questions regarding the roles of cell death and somatic mutation are being pursued in the induction of teratogenesis with MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). In an effort to determine whether all of the affected cells in embryonic target organs (limb buds) are killed after teratogenic exposure to MNNG, DNA damage, repair and mutation are being studied at prenecrotic, necrotic, and postnecrotic intervals.

Another project deals with the role of genetic susceptibility to transplacental carcinogenesis induced by the polycyclic aromatic hydrocarbon, 3-methylcholanthrene, in inbred strains of mice genetically responsive or resistant to these hydrocarbons. Metabolism and DNA binding of 3-methylcholanthrene in maternal, placental and fetal tissues are being studied as correlates to induction of lung tumors in the offspring.

Previous electrophysiological work has shown that lead and cadmium block synaptic transmission by interfering with the actions of calcium leading to transmitter release. Studies with ^{45}Ca have also shown that lead, as expected, blocks the uptake of calcium into presynaptic nerve terminals while cadmium has little effect. From these data it was hypothesized that cadmium must compete with calcium at some intracellular site. The first tests of this hypothesis were based upon the deduction that cadmium would not increase the frequency of miniature endplate potentials in the frog neuromuscular junction, an effect which occurs during exposure to lead and most other heavy metals and is believed to result from an increased concentration of ionized calcium within the nerve terminal. This deduction has been confirmed, which supports the conclusion that the sub-cellular mechanisms of action of cadmium and lead on synaptic transmission are quite different.

Experiments were also conducted to study the toxic effects of heavy metals on olfactory receptor neurons. In these studies the effect of metals was measured on the electro-olfactogram (EOG). The results obtained thus far suggest that olfactory deficits observed following exposure to Zn and Cd may be related to the effects of these metals on the peripheral olfactory structures. Although these acute experiments were carried out with relatively high concentrations of Cd and Zn, it is not unreasonable to expect that much lower concentrations would result in olfactory deficits after chronic exposure.

In another neurotoxicological study, the effects of acrylamide on early peak amplitudes of the somatosensory invoked potential were studied in rats. This research is designed to investigate specifically the relationship between electrophysiological function and acrylamide-induced ataxia. Another aim is to improve the sensitivity of the measurement of alterations in somatosensory evoked potentials and to locate areas of functional deficit responsible for such changes. Consequently, far-field somatosensory evoked potentials, peripheral motor nerve conduction velocity, evoked muscle action potential and gait were measured in acrylamide-treated and control rats. Preliminary results show a reduction in early somatosensory evoked potentials in exposed rats, without significant alterations in the evoked muscle action potential or nerve conduction velocity. The data indicate that acrylamide-induced ataxia is not caused by motor deficits. Instead, damage appears to occur in peripheral sensory nerves or the spinal cord dorsal columns. The evidence also suggests the possibility of damage in the thalamo-cortical areas.

The investigation of effects of lead on the central nervous system included a test of the hypothesis that low level lead exposure during early life may lead to a disruption in the function of the blood brain barrier in neonatal rats. This work involved exposure of the pups to the milk of lead-exposed mothers, followed by lead exposure after weaning. Results thus far have not revealed changes in the ability of choline or tyrosine to cross the blood-brain barrier, in spite of highly elevated blood lead values. A preliminary conclusion is that lead under present conditions does not affect the blood-brain barrier. This finding is important in further studies on the effects of lead on the pharmaco-kinetics of drugs, in particular of d-amphetamine. Work is underway to attempt to learn more about the fate of relatively low doses of d-amphetamine sulfate which stimulate locomotor activity but are not sufficient to result in the more reactive responses observed in higher doses. Findings so far confirm the observation by others that female rodents show protracted behavioral responses under these conditions, most likely due to the longer brain half-life of d-amphetamine.

Bio-organic Toxicology. The study of the biosynthesis of the toxic stress metabolites of the sweet potato has led to the identification of several intermediates between farnesol and ipomeamarone, the major stress metabolite. In the past year an effort was made to determine the source of the oxygen atoms in ipomeamarone. Isotopic oxygen incorporation experiments with $^{18}O_2$, $H_2{}^{18}O$ and [^{18}O]-farnesol showed that all the oxygen atoms in ipomeamarone were derived from O_2 and not H_2O or farnesol. This result predicts a limited number of possible intermediates for furan ring formation and these can be tested by synthesis and feeding studies.

A synthesis of radiolabeled perilla ketone was developed as part of a long-standing interest in the metabolism of 3-furoyl compounds. Although designed as a perilla ketone synthesis, it can be easily altered for synthesis of many alkyl-substituted 3-furoyl compounds.

Biochemical Toxicology. Microsomal proteins were resolved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and transferred to sheets of nitrocellulose. Specific forms of cytochrome P-450 (P-450) were identified on the sheets with the use of an immunoperoxidase staining technique and rabbit antibodies raised to electrophoretically homogeneous forms of rat and human liver P-450. The amounts of each form of P-450 present in microsomal preparations could be detected and quantitated by densitometry at the pmol level. A form of P-450, denoted P-450 PB-B_2, accounted for the majority of the P-450 present in liver microsomes of rats treated with phenobarbital (PB), trans-stilbene oxide, or dimethylnitrosamine. A similar protein was detected in rat lung microsomes regardless of treatment. Another form of P-450, denoted P-450 BNF-B_2, accounted for the majority of the P-450 present in liver microsomes of rats treated with 5,6-benzoflavone, 3-methylcholanthrene (3MC), or 2,3,7,8-tetrachlorodibenzo-p-dioxin. A similar protein was induced by 5,6-benzoflavone in rat lung and kidney. Both forms of P-450 were induced in rat liver Aroclor 1254. Detectable levels of P-450s resembling these two forms were very low in livers of untreated rats, mice, and rabbits, in livers of rats treated with 2-acetylaminofluorene or pregnenolone-16o-carbonitrile, and in rat hearts and brains. Antibodies raised to rat liver P-450s reacted with the inducible rabbit liver P-450s, denoted P-450 LM-2 and P-450 LM-4, and were used to quantitate induction of those proteins. Antibodies raised to human liver P-450 purified from a single patient recognized a protein with identical electrophoretic mobility in liver microsomes prepared from ten different patients and also recognized a protein in the lung microsomes of the two of these patients examined. The portion of total human liver microsomal P-450 which reacted with the antibody varied from 6 to 56 percent among ten different patients. The sensitivity and specificity of these techniques may be of further use in the study of P-450 multiplicity.

Metabolic Toxicology. During the period of this report a number of studies of the biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were carried out. In one such study the lethality of TCDD to the Golden Syrian Hamster was examined. These studies showed the hamster to be the most resistant species to the acute toxic effects of TCDD so far examined. The reason for the increased resistance of the hamster is under study.

TCDD produces a hyperlipidemia in most animal species that have been studied. The effect of TCDD on serum lipid concentrations in guinea pigs was studied.

These studies showed an increase in serum triglycerides, cholesteryl esters and phospholipids as compared to controls. SDS electrophoretograms reveal differences in the C apoproteins in the VLDL fractions. The data from these studies suggested an increased rate of lipoprotein synthesis on administration of TCDD. However, abnormal lipoprotein catabolism as a contributory factor could not be discounted.

There have been reports of mutations in bacterial strains exposed to TCDD. None of the details of these experiments were given in these reports. Therefore, TCDD was examined for mutagenicity to auxotrophs of Salmonella typhimurium. TCDD exhibited no mutagenicity in strains TA 1535, 1537, 1538, 100 and 98 of this organism in the presence and absence of a variety of mammalian activating systems.

Chloramphenicol causes an irreversible inhibition of cytochrome P-450 monooxygenase catalyzed reactions when incubated with rat liver microsomes. A series of studies of the mechanism of the inhibition of P-450-catalyzed metabolism of substrates by chloramphenicol has been carried out. The results of these studies suggest that chloramphenicol causes inactivation of cytochrome P-450-catalyzed reactions by virtue of binding of a chemically reactive metabolite to the cytochrome P-450 apoprotein. This reactive intermediate of chloramphenicol is formed in a cytochrome P-450 monooxygenase catalyzed reaction. The microsomal metabolism of 1,1,2,2-tetrachloroethane was also examined during the period of this report. These studies showed that liver microsomes, or a purified cytochrome P-450 monooxygenase system, is capable of metabolizing 1,1,2,2-tetrachloroethane to dichloroacetic acid. The data appear to rule out dichloroacetaldehyde as an obligatory intermediate but are consistent with an acyl chloride intermediate.

Naturally Occurring Toxicants. Search for the specific fungal metabolic agent causing equine leuko-encephalomalacia (ELEM) has continued. Although Fusarium moniliforme-infected corn is now well established and recognized as the specific nutritional source of intoxication, the nature of the toxicant is still elusive. There is new evidence to suggest that the fungus is also the source of toxin causing swine pulmonary edema (SPE) and somewhat different disease syndromes in other animal species.

Two different metabolites were obtained from F. moniliforme. The first of these was identified as 4-ethylguaiacol, a compound that had been previously located in several places in the environment. 4-Ethylguaiacol is relatively non-toxic to laboratory animals and equines. This is the first report of its occurrence as a fungal metabolite. The second metabolite recently isolated from both fungal liquid shake cultures and from fungus-contaminated corn seems to be a novel compound with acute toxic properties for laboratory rodents. It also appears to have significant antibiotic properties against certain bacteria in vitro. A preliminary test in which crude toxin was injected into a donkey failed to reveal any significant effect on this equine species. Intensive efforts to characterize this metabolite further are underway.

Considerable research effort has gone into further studies on the mechanism of action of perilla ketone (PK), a toxic metabolite of the common mint plant, Perilla frutescent. Particular attention has been given to comparing parameters of its covalent binding to liver microsomes with that previously reported for 4-ipomeanol, chemically related stress metabolite of the fungus-infected sweet potato. Recent work has demonstrated again that PK is metabolically activated by

hepatic microsomal enzymes to an alkylating species that binds rapidly to microsomal macromolecules through a cytochrome P-450-dependent mechanism. Kinetics of the PK covalent binding suggest that the mint metabolite is more reactive than 4-ipomeanol. There is some evidence to suggest that the reactive species for both PK and 4-ipomeanol may be an unstable epoxide which thus far has not been isolated. Pretreatment of rats with the inducers phenobarbital and 3-methylcholanthrene increased the hepatic microsomal P-450 and covalent binding of PK in vitro. However, the induced P-450 has a lower specific activity toward PK binding than that of control animals. Pretreatment of rats with PK decreased the P-450 content and covalent binding of PK to a greater extent than did 4-ipomeanol pretreatment. Both pretreatments similarly decreased the specific activities of the P-450 when compared to controls. The former findings are in contrast to work by other workers in which a direct correlation was obtained between cytochrome P-450 content induced by phenobarbital and 3-methylcholanthrene and the amount of 4-ipomeanol bound in vitro.

Further studies have confirmed the marked susceptibility of ponies to PK toxicity. Intravenous doses of 18 mg/kg produced severe respiratory disease which became evident within a few hours and gradually more severe (dyspnea) until death or euthanasia at six days. At necropsy, the lungs were found to be edematous with alveolar and interstitial edema and other features which histologically resembled the picture seen in acute pulmonary edema and emphysema of cattle (ABPE). Various pulmonary, cardiac, and circulatory parameters were monitored during the disease course. There was a pronounced increase in respiratory depth and rate, minute volume, flow rates, work of breathing, intrapleural pressure change, and pulmonary arterial pressure. There was a decrease in tidal volume, dynamic compliance, and functional residual capacity. At 6 days after dosing the PaO_2 was low, the maximum change in pleural pressure during tidal breathing was elevated, and the work of breathing per minute was greatly increased. Comparisons of the above findings with data obtained in ponies given approximately 200 mg/kg 3-methylindole, (3MI) a metabolite of l-tryptophan, suggest that the two agents produce somewhat different syndromes in equines. PK causes acute pulmonary edema without marked bronchiolitis, whereas 3MI administration results in a necrotizing bronchiolitis which is functionally and morphologically an obstructive pulmonary disease.

Harvard University

Radiobiology and Experimental Carcinogenesis. The research program emphasizes studies at the cellular and molecular levels in an integrated approach to the investigation of the lethal, mutagenic and carcinogenic effects of radiation and chemical agents in mammalian cells. In a number of studies, radiation is used as a model for the action of many chemical agents at the level of DNA. One focus is on the interactions between radiation and chemical agents in particular agents and factors which may modify the mutagenic and carcinogenic effects of radiation. Another is on the role of genetic predisposition to these effects in human populations.

Specific areas of research include studies of: 1) The induction of malignant transformation in mammalian cells, especially the role of non-carcinogenic secondary factors which may modify transformation induced by DNA damaging agents; 2) Mutagenesis and cytogenetic changes in mammalian cells; 3) Genetic susceptibility to spontaneous and induced cancer, by examining somatic cells from individuals with either recognized genetic disorders or from families associated

with an increased risk of cancer; and 4) The role of specific molecular DNA repair processes in the expression of the cellular effects of DNA damaging agents.

The most recent series of experiments have focused in three areas; (1) the study of the influence of dose rate on lung tumor induction by ^{210}Po (48); (2) studies of interactions between radiation (^{210}Po and radon gas) and BP in the induction of experimental lung cancer; and (3) a study of the role of cell proliferation in lung tumor induction by sequentially administered ^{210}Po and BP (98). For the dose rate studies, animals were exposed to ^{210}Po in varying numbers of weekly intratracheal instillations such that they received 90% of the total dose over a time period ranging from 8 to 120 days. Three groups of animals were exposed to total lifetime radiation doses of 2.4, 24 and 240 rads. The tumor incidence appeared to decline as the dose rate was protracted in the high exposure group (240 rads lifetime dose), but to be enhanced with protracted exposure following the total dose of 24 rads.

Biochemical and Environmental Toxicology. The principal current research activities are organized into two broad areas: 1. genetic toxicology; and 2. cellular and biochemical toxicology. The specific aims within each of these broad areas involve the following specific projects: 1. Genetic Toxicology; 2. Mechanisms of action of chlorinated aromatic compounds; 3. New Model Systems in Toxicology: Cultured epithelial cell differentiation and response to toxic agents; 4. Heavy Metal Toxicity; 5. Mechanisms of Action of Tumor Promoters; 6. New Model Systems in Toxicology: Studies on differentiated function mammalian cells in long-term culture; 7. Adenylate cyclase and cytochrome P-450 targets; and 8. Sex hormone actions and metabolism in male target tissues.

It was found that keratinocyte lines derived from human epidermal and squamous cell carcinomas respond in a dose-dependent fashion. The reversion events can be either a GC → TA transversion at either the first or second base pair of a ccc codon(proline). A third reversion event has been detected which does not affect the proline codon and efforts are being made to determine if this event is an intra- or inter-genic suppressor mutation. The ability of this mutation to revert to wild-type by at least three different pathways provides a molecular explanation for the responsiveness of this strain to many mutagenic agents.

Respiratory Biology and Inhalation Toxicology. A new probe was developed to study the maturation of pulmonary macrophages in relation to functional activity of these cells. It was found that pulmonary macrophages of mice and hamsters have a specific antigen on their surface which is absent from non-elicited peritoneal macrophages and macrophages in other tissues. This antigen is found in both inbred and outbred strains of mice. Antibody to the antigen is found in the normal sera of rabbits from a variety of sources, and higher titers can be induced in rabbits or guinea pigs by inoculation with pulmonary macrophages.

Immunoferritin was used in ultrastructural studies to show differences in antigen distribution on pulmonary macrophages of different stages of differentation. Ultrastructural studies indicate that the amount of the specific antigen on the surface is correlated with the number of pseudopods and with the number of phagosomes, suggesting that a greater degree of antigen is expressed on stimulated cells. The antigen is present in significantly lower quantities on pulmonary macrophages of germ-free mice as opposed to conventional mice, indicating that this antigen may be expressed in response to environmental factors. Exposure of mice to carbon resulted in an in vivo increase of macrophages in the lung and an increase in macrophages without antigen.

A technique was developed for measuring aerosol retention by autoradiography which allows the anatomical site of deposited particles to be determined. In this procedure, a slice of dried lung which has previously breathed a ^{99m}Tc-labelled aerosol is put in contact with X-ray film for 6-12 hours (one to two half-lives). In addition to a 140 KeV gamma ray, ^{99m}Tc also emits low energy electrons upon decay (1.6, 1.9, and 119 KeV). Since these electrons are strongly ionizing they are the predominant source for film exposure. Typically, 10^8 gamma rays/cm^2 film are required for unit density whereas only 10^7 electrons/cm^2 are required. The short range of the electrons (90 μm in film) results in a crisp image of the distribution of radioactivity over the face of the slice. The developed film is a high-resolution, compact record of retention site with anatomical features readily visible. To convert this record into quantitative information, a correlation of anatomy with film density has been worked out using a computer-assisted scanning microdensitometer.

The extent to which exercise potentiates ozone induced pulmonary edema and mortality has been examined. Mice subjected to five hours of continuous light exercise during exposure to 1.0 to 3.0 ppm O_3 showed a marked mortality compared with animals exposed but not exercised. The LC_{50} at six hours for non-exercised animals was 9.0 ppm. I^{131} albumin recovered in lavage showed a direct relationship with O_3 concentrations for both exercised and non-exercised groups. Exercised groups showed I^{131} accumulation in lung airspace similar to that of non-exercised groups, but at far lower O_3 exposure concentrations. It is concluded that exercise superimposed on O_3 exposure potentiates the toxic effect of pulmonary edema accumulation and consequent mortality.

The effect of sulfur dioxide on another response of the respiratory system, namely pulmonary macrophage function was also studied. The effects of exercise were explored by measuring the rate of particle ingestion by pulmonary macrophages *in vivo* with and without SO_2 exposure. Hamsters breathed 50 ppm SO_2 in a Plexiglass box containing a variable speed treadmill. One, 24, and 48 hours following exposure, the *in vivo* phagocytic rates or pulmonary macrophages were measured by monitoring the uptake of radioactive colloidal gold (^{198}Au). In addition, the number and size distribution of cells harvested by pulmonary lavage were determined to show the extent of the inflammatory response.

When hamsters exercised without SO_2 exposure, or were at rest and exposed for four hours to 50 ppm SO_2, no significant reductions in macrophage endocytosis were observed at 1, 24 and 48 hours post-exposure. However, in hamsters exposed to SO_2 while exercising continuously on a treadmill, endocytosis decreased from 64% to 41%. One day after exposure to SO_2 while exercising, the percent ingested had returned to control values. Thus, the alterations in macrophage function caused by SO_2 and exercise were immediate and short-lived, with approximately normal phagocytic indices returning by 24 hours.

Environmental Epidemiology. The Environmental Epidemiology core of the Environmental Health Center is located principally within the Department of Epidemiology. The research of this unit has two principal objectives: (1) to understand the etiology of those diseases of which the etiology is currently unknown and which are the major causes of mortality in the United States today - neoplastic disease has been selected as the principal focus of this research, and (2) to evaluate the long-term effects of exposure to substances present in the environment of the U.S. population.

Although firefighting involves obvious health hazards, previous studies of mortality and morbidity in firemen have produced inconsistent evidence for an increased risk of mortality from cardiovascular disease, respiratory disease, cancer and accidents. Mortality experience since 1915 has been examined in 5,655 Boston firefighters, comprising all male members of the fire department with three or more years of service. The observed cause of death as stated on the death certificates of 2,470 deceased firefighters has been compared with the numbers expected based on rates for the male population of Massachusetts and of the United States of America. Among all firefighters, deaths from all causes were 91% of expected. The standardized mortality ratio (SMR) was markedly reduced (less than 50) for infectious disease, diabetes, rheumatic heart disease, chronic nephritis, blood diseases and suicide. The SMR was 86 for cardiovascular deaths, 83 for neoplasms, and 93 for respiratory deaths. The SMR for accidents was 135 for active firefighters and is strongly influenced by strict entry selection procedures, ethnic derivation, and sociocultural attributes of membership. While excessive morbidity has been demonstrated in firefighters, there does not appear to be a strong association between this occupation and cause-specific mortality.

No increase was seen in the total number of cancer deaths in a fluoroscopy cohort in comparison with an unexposed cohort [relative risk (RR)=0.8]. Elevated risks of mortality from stomach cancer (RR=2.3), rectal cancer (RR=3.8), breast cancer (RR=1.2), lung cancer (RR=1.8), and leukemia (RR=1.2) were observed, but none was statistically significant and all were based on very small numbers of deaths. Increases were balanced by decreases in genital cancer (RR=0.2), pancreatic cancer (RR=0.9), lymphoma (RR=0.6), and all other cancers (RR=0.1). Average cumulative absorbed doses were estimated at 110 rads for the lungs, 33 rads for the trunk, 13 rads for the active bone marrow, and 7 rads for the stomach. These findings suggest that the carcinogenic effect of multiple low-dose X-ray exposures is not greater than that currently assumed.

Because of the association between radiation exposure and breast cancer incidence noted in the study described, a study of a larger group of women and a group of men has been initiated. Cohorts of approximately 6,000 women and 2,000 men who were treated for tuberculosis in Massachusetts between 1930 and 1950 have been identified and traced as to current vital status. A medical questionnaire is being sent to persons found to be alive.

The Six-Cities Study, led by Dr. Benjamin Ferris, has completed seven years of data collection. The third round of examinations of adults has started. There has been some attrition of the adult population due to deaths, moving away, and refusals. Numbers, however, still seem to be adequate. Data collection on the children has continued with somewhat lower rates of attrition.

Cross-sectional analysis of the health effects and associated concentrations of sulfur oxides and particles are nearly completed. The concentrations measured in the various cities do not appear to be having a very marked effect. There may be a small effect in Steubenville, but further analysis and data are needed to confirm this.

Earlier observation that the use of gas cooking stoves was associated with chest illness before age two has not been confirmed. It appears that the association is quite sensitive to the assumptions included in the model, plus there is a significant cohort effect. The observation that there is a small reduction in pulmonary function in such children still remains.

Additional measurements have been made of pulmonary function of children before, during, and after "alerts" in Steubenville. The decline associated with the "alerts," was present. The pulmonary function was plotted against the concentrations of air pollution for the 24 hours before the measurement of pulmonary function and it appeared that there was a linear decline of FVC and $FEV_{.075}$ with increased concentrations of TSP and SO_2. The change is statistically significant, but small. The medical significance still needs to be resolved.

Air monitoring has continued at the various sites by means of various central locations. The spatial network of indoor/outdoor monitoring has been terminated. Some of the samples collected have been analyzed by means of neutron activation. The interpretation of these results is still in progress. E.P.A. dichotomous samplers are being run in various areas. This will give more information on two other particle size cuts - one below 2.5 μm, the other 2.5 um to 15 um. These are referred to as the fine and coarse fractions.

University of California at Berkeley

The Department of Biochemistry at the University of California, Berkeley, has established a broadly based program in environmental metagenesis. Ongoing projects in the center range from basic research in the area of nucleic acid metabolism to improvement of the widely used Salmonella mutagen testing system and the development of a human dosimeter to assess DNA damage. The major research objectives of the center include:

Mutagenesis - A new Salmonella frameshift tester strain, TA97, has been developed with a run of 6 C's at the site of a +1 addition. This is considerably superior to the old TA1537 tester strain and is designed as a replacement.

Improvements in the Salmonella test have been designed to detect two major groups of carcinogens which have shown up as "false negatives" in the standard test system. The lack of response of one of these classes may be due to the fact that the active forms of the carcinogens may be radicals which have very short half-lives, and which can cause DNA damage by causing a lipid peroxidation chain reaction. To facilitate the interactions of the active forms with the test bacteria, the Ames group has developed two tester strains which detect some of the oxidants caused by lipid peroxidation, such as hydroperoxides and oxygen radicals. One strain contains a run of 5 AT pairs at the site of a +1 frameshift mutation, and the other contains AT pairs in a base pair substitution.

A second class of "false negatives" in the Salmonella test are natural carcinogens present in the human diet as glycosides. In order to detect these carcinogens, one needs a model for the metabolism of the bacteria in the human colon, as some glycosides are split by these bacteria to liberate mutagens. The Ames group has now developed such a model that works quite well for this whole class of compounds. They have made an enzyme preparation, which they call fecalase, by sonicating human feces (which is made up of bacteria to a large extent) and have shown that fecalase contains a wide variety of enzymes splitting sugars from glycosides. By adding fecalase to the Salmonella test, they have also shown that many different naturally occurring glycosides of mutagens (flavonoids, anthraquinones, cycasin, etc.) now show up as mutagens.

In addition, they have characterized several human repair enzymes, including apurinic (AP) endonuclease, UV endonuclease, uracil DNA-glycosylase, and an enzyme that specifically inserts purines into apurinic sites in DNA ("purine insertase"). This latter enzyme has been shown to be inhibited by caffeine. They have also shown that xeroderma pigmentosum strains of complementation group D are lacking an AP endonuclease isozyme with a high affinity for AP sites. Finally, they have shown that, as is the case in E. coli, human cells have two AP endonucleases that can act together to excise deoyribose phosphate from AP DNA.

Nucleic Acid Replication and Metabolism

The Tjian laboratory has been a leading group in the investigation of the origins of DNA replication in eukaryotes. Their primary objective this past year was to develop systems for systematically mutagenizing specific regulatory sequences, such as eukaryotic origins of DNA replication and transcriptional promoters. They have succeeded in using a variety of recombinant DNA techniques in concert with special emphasis upon in vitro mutagenesis to generate a large collection of origin and promoter mutations. The functional analysis of the mutant origin sequences was carried out in a specialized transfection system. Deletion mutants in eukaryotic promoter sequences were analyzed by an in vitro system. They intend to extend their work by utilizing different mutagens to create single site mutations to analyze further the influence of these specific regulatory sequences on the control of gene expression.

Studies in the Penhoet laboratory have examined the metabolism of modified nucleosides in transfer RNAs. Their studies have led to the characterization of enzymes responsible for the biosynthesis of pseudouridine in prokaryotes, yeast, and mammalian cells. These enzymes appear to be homologous in all species examined and demonstrate unusually broad specificity, introducing pseudouridine residues into the anticodon region of almost half the +RNAs in a particular cell.

The Penhoet group has also been analyzing eukaryotic gene structure. Studies of fructose 1,6-diphosphate aldolase genes are well under way. These studies will define the structure, organization, and expression of this multigene family.

In the Dekker laboratory the four major RNases of human urine, RNases HU_{A-d}, were subjected to glycosidase digestion followed by SDS-polyacrylamide gel electrophoresis and activity staining. Using this novel procedure, RNases HU_{A-C} were shown to be glycoproteins of the complex type while RNase HU_D, though containing ca. 10% sugar by weight, was apparently unaffected by glycosidase digestion. Evidence was obtained suggesting that RNases HU_{A-C} share a common polypeptide.

Eukaryotic Cell Structure

Mutagenesis and teratogenesis may occur by defects in mitotic mechanisms that control the separation of sister chromatids during cell division. Work in Cole's laboratory is aimed at an understanding of the control of cell division, particularly in the dynamics of chromatin structure, and the assembly of the microtubules (and other components) of the mitotic spindle. The major accomplishments this year included a consolidation of last year's discovery that ATP enhances microtubule assembly directly. The binding coefficient between ATP and tubulin was measured and, by use of an affinity label, the ATP binding site was shown to be

distinct from the two previously established GTP sites. ATP seems to be an effector of conformation, since it is incorporated but not hydrolysed during microtubule assembly. Kinetic studies showed that ATP enhances the nucleation of assembly and the elongation as well.

The Schekman laboratory is studying the eukaryotic cell surface and its assembly. They have developed techniques for the isolation of temperature-sensitive mutants of yeast that are simultaneously defective in secretion and cell-surface growth (sec mutants). This procedure revealed 23 complementation groups that are required for the movement of at least two secretory enzymes and two plasma membrane permease activities through a series of membrane-bounded organelles on a pathway that leads to the cell surface. The isolation technique also produced a novel class of mutants that are defective in the formation of active secretory enzymes, possibly due to a failure in translocation of proteins into the endoplasmic reticulum. Double-mutant analysis and studies on the compartmentalized assembly of glycoprotein oligosaccharide chains have allowed an assessment of the order in which the sec gene products are required, the sequence of carbohydrate muturation steps, and a pathway of secretory organelles.

Mutation and Evolution

Wilson's laboratory seeks to understand the role of mutations in the evolutionary process. The work is concerned not only with point mutations in structural genes, but also with mutations causing rearrangement of genes. The two gene systems under study are mitochondrial DNA and globin genes in primate species. The highlights are as follows:

Chemical sequencing of a cloned segment of mitochondrial DNA from humans and four species of ape has shown that ninety percent of the point mutations accepted by this genome are transitions. The proportion of silent changes in the protein-coding regions is high, which indicates the operation of strong functional constraints on m+DNA codons.

High resolution mapping of endonuclease cleavage sites in m+DNA from 110 humans shows a high rate of substitution in +RNA genes, and indicates that there may be more m+DNA variation among aboriginal Australians than in the rest of the human population in the Old World. This suggests the possibility of paternal contributions and recombination with the maternal genome during the history of human diversification.

Restriction studies of genetic polymorphisms in the zeta globin region of 50 humans has given a more detailed picture of the types and locations of rearrangements than is available for any other region of the human genome. The most notable discovery is that of length polymorphism in the introns of both the zeta 1 and zeta 2 genes.

Medical College of Wisconsin Freshwater Biomedical Center

The polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are extremely toxic chemicals that have been detected in freshwater fish in areas of South Vietnam that were sprayed with 2,4,5-T and in military test areas in the United States. Recently, PCDDs containing four to eight chlorine atoms were detected at the ppt level in fish samples from the Tittabawassee River

and Lake Ontario. PCDFs containing four to seven chlorine atoms were found at the ppb level in all fish samples from the Ohio, Connecticut and Hudson Rivers and Lake Michigan.

Exposure of fish to TCDD in a model ecosystem has shown that they accumulate the compound to a higher concentration than ambient water. TCDD residue concentration and in fingerling catfish were 2,000 - 28,000 times greater than the water concentration and in Gambusia 9,000 - 63,000 times greater. No metabolites of TCDD were found in either fish. Evidence that TCDD is concentrated through the food chain has also been shown. In a two-step food chain using mosquito larva and fish the concentration of TCDD in fish was 350 times greater than in the same fish which did not have mosquito larva as a food source.

Research is currently being pursued to establish whether fish are in fact more sensitive than rodents to permethrin or whether the difference in lethality is associated with differences in disposition, rate of metabolism or both. Work is being conducted concerning the comparative pharmacokinetics and metabolism of permethrin in fish and mice. The half life of permethrin in fish may be longer than in mammals, and this could answer some questions concerning selective toxicity. Other explanations of the selective toxicity may be that fish are less able to metabolize permethrin or that they may metabolize permethrin in a different manner than mammals, possibly creating a metabolite more toxic than the parent compound. An interesting finding suggestive of additional differences in the toxicity of permethrin in trout and mice is the observation that trans-permethrin is more toxic to trout than the cis isomer while the opposite is true in mice. Recent studies utilizing liver preparations from rainbow trout indicate that the trans isomer is more susceptible to hydrolysis than the cis isomer. Since this latter finding is similar to that reported for rats, the reason for the difference in the toxicities of the cis and trans isomers in trout and rodents, as well as the overall mechanism of selective toxicity of permethrin in these species, does not appear to be based wholly on inherent esterase activity. This problem has in reality been approached from opposite ends of the spectrum and studies on both "target organ" sensitivity as well as differential biotransformation of permethrin have been initiated.

Considerable evidence favoring the concept that epinephrine and theophylline exert their positive inotropic action upon cardiac muscle by elevating tissue levels of 3',5'-AMP via different mechanisms has been presented. Studies have indicated that the mechanism of action of epinephrine on muscle tissue appears to be related to activation of glycogenolysis but involving only those steps prior to the energy yielding reactions of the Embden-Meyerhof pathway. Experiments have been designed to investigate this area of interest. The isolated perfused turtle heart has been selected as the experimental object because it has certain metabolic characteristics which indicate that it may be able to answer the specific questions asked.

a) The turtle heart can be stimulated by the administration of epinephrine or theophylline.

b) Dichloroisoproterenol (DCI) can effectively block the positive inotropic action of epinephrine or theophylline. A total dose of 160 ug of DCI perfused through the turtle heart over a period of one hour provided greater blockage against the action of epinephrine than against the action of theophylline. This difference in blocking action is highly significant. It may simply mean that

the large number of theophylline molecules involved in this competition effectively displace the DCI molecules from their receptor attachment. Further studies are indicated involving higher doses of DCI to clarify this point. An entirely different suggestion is that theophylline has some special function not shared by epinephrine which contributes to its positive inotropic action.

Preliminary experiments have indicated that the metabolism of the isolated turtle heart is very similar to that of the isolated frog heart. It may function entirely on the energy obtained from glycolysis for long periods of time or one may poison the glycolytic energy-producing steps with iodoacetic acid; and the heart will derive its energy for contraction from oxidative metabolism.

In recent work, it was shown that triethyltin exerts a profound effect on the oxygen affinity of hemoglobins (cat and rat) to which it is bound. It has no effect on the oxygen affinity of other hemoglobins, including those of trout and carp but it has a general hemolytic effect on the red cells of aquatic and terrestrial species. This effect is associated with the selective inhibitory effect of triethyltin on hexokinase and on red blood cells Na^+/K^+ ATPase. Red cell hexokinases of aquatic species are of particular interest because they demonstrate wide differences in sensitivities to uM concentrations of triethyltin. The sensitivities of hexokinases in other tissues are also of interest.

This work is expected to provide data necessary to understand the ways in which alkyltin interacts with biological systems in aquatic and terrestrial species to produce toxic effects. Its aim is tc identify the sensitive systems and to understand the molecular mechanism of action. It focuses upon effects on the red blood cell as a simple model cell system, because of prior knowledge that triethyltin enters the red cell, binds specifically to certain hemoglobins (e.g., cat Hb) and alters the oxygen affinity of blood. In addition, it affects the stability of the erythrocyte.

Fish are susceptible to the development of cutaneous fungal infections with several genera of organisms in the family Saprolegniaceae. A model of experimental cutaneous fungal infection in rainbow trout have been developed and the defense mechanisms employed by the fish to prevent this type of infection. These studies indicate that inflammatory cells, particularly neutrophils, may play an important role in this process. The present work will attempt to elucidate further the host-parasite interactions involved in these infections and will also address how they are altered by various physical or chemical factors.

There are two major advantages to the use of fish in this kind of study: (a) they are poikilothermic so that temperature effects on host defense and particularly neutrophil function can be studied in vivo over a much wider range of temperatures than in mammals; (b) they naturally accumulate from their environment high levels of certain pollutants such as polychlorinated biphenyls (PCBs) which have been demonstrated to be immunosuppressive in other systems. Studies of the effects of these pollutants on host-parasite interactions in a type of infection that also occurs naturally may reflect processes which are presently occurring among wild fish and other animals as well.

A question of phylogenetic interest is whether interferon from one species will bind to interferon cell receptors of species from different orders or classes. With suitably sensitive assays of interferon developed, cells bound to bromoacytl cellulose columns can be used to absorb interferon and relative efficiency of

binding; and elution of interferon from different cells can be studied. A second major study of interferon action is being investigated involving the critical events during the viral growth cycle of IPN virus when interferon inhibition occurs. Since virus replication of IPN virus can be slowed by nearly a factor of 2 by shifting down from 24^{0} to 15^{0}, it should be possible by carefully executed temperature shifts up and down to determine whether viral uncoating, RNA replication, viral enzyme formation, or virus maturation are affected by interferon. Investigations into the molecular mechanism of the antiviral action of interferon are focusing on the induction of an oligonucleotide inhibitory of protein synthesis in interferon-treated cells. It has been reported that when double-stranded RNA is added to extracts of interferon treated mouse L cells, an enzyme is activated which utilized ATP to form an inhibitor of protein synthesis. This inhibitor was characterized as pppA $(2'p5'A)_{1-4}2'p5'A_{OH}$ or 2'5'oligo(A). The enzyme that synthesizes this oligonucleotide has been identified in other animal species but it is not yet reported in fish cells. The 2'5'oligo(A) inhibits protein synthesis by activating a nuclease which prevents mRNA from being utilized for protein synthesis. Possibly the 2'5'oligo(A) may represent the mechanisms of inhibition of virus replication in interferon-treated cells.

The insoluble proteins of the cornea, particularly the stroma collagens, have recently been studied in some detail. Corneal soluble proteins, on the other hand, have attracted minor interest and consequently little is known about their distribution and possible functions in the component layers of the cornea.

Studies have shown that the corneal epithelium contains 20 or more distinct soluble proteins, none of which are of serum origin. The corneal stroma contains somewhat fewer distinct soluble proteins, the majority of which are of serum origin. Immunochemical analyses have demonstrated that each component layer of the cornea has one or more specific antigen proteins though some antigens are shared by two or more layers. In addition to these specific antigens, the cornea contains high levels of soluble antibody proteins which are derived from serum, notably IgG, IgA and IgM.

Recently, the major soluble protein of the bovine corneal epithelium have been isolated and characterized. This protein was found to have a molecular weight of 54,000 and has thus been termed BCP54. In addition to being the principal soluble protein of bovine corneal epithelium, it is also a major stromal protein. Its presence in the endothelium, however, is uncertain. This protein seems to be specific to the cornea and appears to be immunochemically related to a similar protein in the human cornea. It is now being explored in fish.

In previous years, the ultrastructural and metabolic aspects of corneal transparency in marine vertebrates have been investigated. Particularly interesting is the cornea of the marine elasmobranch (Squalus acanthius) as it compares with the cornea of the marine teleost (Myoxocephalus octodecimspinosus). The elasmobranch cornea, a non-swelling tissue, is both structurally and metabolically distinct from the teleost cornea - a tissue which is hydrophilically, structurally and metabolically similar to the mammalian cornea. The proposed soluble protein studies will further understanding of the development of corneal transparency from a phylogenetic point of view and further knowledge of specific adaptations which have insured corneal transparency in extremely hostile osmotic environments.

The effects of various types of inducing agents on the hepatic microsomal monooxygenase (MO) system of rainbow trout were examined and compared to the induction profiles observed following pretreatment with either 3-methylcholanthrene- or phenobarbital-type inducers. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) elevated ethoxycoumarin- and ethoxyresorufin-O-deethylase activities (ECOD, EROD) as well as cytochrome(s) P-450 content, but had no effect on benzphetamine-N-demethylation (BeND). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of solubilized microsomes from TCDD-pretreated fish demonstrated the intensification of a protein band with a molecular weight of 57,000. Although the magnitude of induction was not as great, isosafrole pretreatment of rainbow trout resulted in an induction profile similar to that observed following TCDD administration to these animals. Neither Kepone nor mirex had a stimulatory effect on any of the measured parameters and neither caused a change in the protein profile following SDS-PAGE of solubilized microsomes. These results suggest that fish respond differently from mammals to these inducers of the hepatic microsomal MO system.

The distribution and metabolism of the cis- and trans-permethrin isomers were studied in rainbow trout to evaluate the role of these parameters in the differential toxicity of permethrin to fish and mammals. Both [^{14}C]permethrin geometrical isomers were readily taken up and eliminated by rainbow trout. Elimination half-lives for [14C]permethrin residues in trout tissues, with the exception of fat, were in the magnitude of hours. High concentrations of a polar metabolite were found in bile within 4 hrs of cis- and trans-permethrin exposure. Analysis by β-glucuronidase treatment, analytical thin-layer chromatography, and gas chromatography-mass spectroscopy indicated that the metabolite was the glucuronide conjugate of 4'-HO-permethrin.

Oregon State University Marine and Freshwater
Biomedical Center

Effects of polychlorinated hydrocarbons on aflatoxin B_1 (AFB_1) carcinogenesis in rainbow trout was determined. It was found that the timing of PCB administration, relative to AFB_1 exposure, is very important in determining the effects of PCBs. Simultaneous dietary exposure of PCB (Aroclor 1254) and AFB_1 resulted in a significant decrease in the incidence of hepatic tumors. Exposure of trout embryos to AFB_1, followed by dietary exposure to PCB, showed neither promoting nor inhibiting effects of PCBs on previously initiated tumors. Exposure of embryos to PCBs prior to AFB_1 challenge, by feeding PCB to gravid females, resulted in a marked increase in tumor incidence over non-PCB exposed controls. The dietary levels of Aroclor 1254 that were routinely fed cause significant mixed function oxidase (MFO) enzyme induction. These initial experiments indicate that, (1) simultaneous exposure of PCB and AFB_1 cause a reduction in AFB_1 metabolism, probably through competitive inhibition, and a reduction in tumor initiation; (2) PCBs do not promote or inhibit preciously initiated tumors; and (3) prior exposure to PCBs and MFO induction may result in increased AFB_1 metabolism and increased carcinogenicity.

Dietary exposure of rainbow trout to tansy ragwort pyrrolizidine alkaloids (PAs) resulted in both acute and chronic pathologic effects. A level of 100 ppm in the diet caused severe liver megalocytosis, necrosis and fibrosis and high mortalities after only two months. Fish that were returned to a control diet after two months on 100 ppm PA failed to recover from the initial toxicity and liver lesions were still present 10 months later. There were no increased mortalities

in the groups fed 20 ppm PAs but livermegalocytosis, necrosis, fibrosis, veno-occlusion and vascular changes in the kidneys were present. *In vitro* metabolism studies showed that trout metabolize the PAs to the corresponding toxic pyrrole compounds as efficiently as do rats. The trout thus appears to be a suitable animal model for PA research.

Feeding trials have established the carcinogenicity of aflatoxicol (AFL) in both rainbow trout and rats. Its potency appeared to be about one half that of AFB_1 in both trout and rats. Other feeding experiments also established the carcinogenicity of aflatoxin Q_1 (AFQ_1) for the first time in rainbow trout. This *in vitro* metabolite, from human liver preparations, was about 100 times less potent than AFB_1. Small quantities of aflatoxin P_1 (AFP_1) were obtained from Dr. Gerald Wogan of MIT. Trout embryos were exposed in two ways (5.0 ppm AFP_1 for 30 minutes) and (1.0 ppm for 24 hours) but neither exposure produced tumors 12 months later. Although these were not definitive tests, they indicate that AFP_1 is either non-carcinogenic or of very low potency, supporting Ames mutagen assay data for this metabolite.

Fingerling rainbow trout were fed diets containing one of the following compounds: flavone, tangaretin, β-ionone, quercetin, α-naphthaflavone and indole-3-carbonol, for three months prior to dietary exposure to 20 ppb AFB_1 for two weeks. After AFB_1 exposure, the trout were fed control diet and held to permit tumor development. At a recent nine-month sample, two of these compounds, γ-naphthaflavone and indole-3-carbonol, appeared to have inhibited tumor initiation since tumor incidence was significantly lower than in positive control animals. The experiment will be determined at 12 months and the results correlated with MFO determinations that were assayed before AFB_1 exposure.

Previous experiments suggested that cyclopropene fatty acids (CPFA) depress the MFO system and AFB_1 metabolism. An experiment was conducted in trout to measure the effect of CPFA on the induction of the MFO system by a known inducer, Aroclor 1254 (PCB). Dietary PCB (100 ppm) fed for 9 weeks markedly induced the 7-ethoxyresorufin-O-deethylase, 77-fold; 7-ethoxycoumarin-O-deethylase, 7-fold; benzo(a)pyrene monooxygenase, 45-fold and cytochrome P-450, 2-fold above control levels. Addition of 50 ppm CPFA to the PCB diets reduced the induction of all parameters by approximately 50%. The activity for these parameters in CPFA control trout was lower than in controls except for benzo(a)pyrene monooxygenase which was similar to control levels.

A dose-response experiment on the effect of CPFA doses from 0 to 600 ppm on the MFO system in yearling trout revealed that a dose of 150-300 ppm produced the maximum effect. Correlation coefficients indicated a dose-response for cytochrome P-450, nmole/mg microsomal protein (-0.9335); NADPH-cytochrome c reductase, nmole/mg microsomal protein/min (-0.8869). The negative correlation coefficient indicates a decrease with increasing CPFA dose. Low levels of dietary CPFA (50, 150, and 300 ppm) caused an increase in benzo(a)pyrene hydroxylase but high levels (450 and 600 ppm caused a decrease in the activity.

In another experiment the acute effect of 300 ppm CPFA on the MFO system was measured as a function of time up to nine days. In general, dietary CPFA caused a decrease in cytochrome P-450 and cytochrome b_5 content as well as a decrease in most MFO enzyme activities. Cytochrome P-450 content significantly decreased in CPFA-fed trout by day four and by day nine there was a 50% decrease in P-450 content. Cytochrome b_5 also decreased but to a lesser extent than P-450. NADPH-cytochrome c reductase decreased 25-30% in activity by day nine. Benzo(a)pyrene

hydroxylase increased substantially from day three to nine. Usually the pattern in MFO profile was established by days seven-nine and remained for a few weeks until liver damage became excessive.

Duke University Marine Biomedical Center

Duke University and the University of North Carolina System have created an Oceanographic Consortium. This new Consortium brings together oceanographers from throughout the State of North Carolina and creates an institution which will rank in size with the largest oceanographic institutions in the world. The Consortium will take delivery on its newest research vessel, R/V Cape Hatteras in 1981. The new ship is designed for coastal and estuarine research projects. It will be of use to members of the Marine Biomedical Center whose questions are best answered by shipboard experimentation.

Studies are being conducted on the chelation and transport of metals. The studies have concerned the possible influence of ligating pollutants and salinity on iron bioavailability. Iron is present in seawater in trace amounts (10ppb) and is concentrated by a factor of 10^6 by marine plants. In seawater, iron is found as insoluble ferric hydroxide; and aquatic organisms must consequently have developed mechanisms for solubilizing and transporting iron. The bioavailability of iron is made possible by microorganism-produced chelators called siderophores-phenolates and hydroximates which influence iron availability. Exploratory experiments have been done which test the hypothesis that certain organic and inorganic pollutants in natural waters may influence the bioavailability of iron and other trace metals by altering the mechanism of iron transport. This harmful effect may be quite apart from known toxic mechanisms for these pollutants. The thrust of these experiments was to determine if a foreign chelator may influence the chelation of iron by siderophores or its release from the siderophore to the organism. The experiments have clearly demonstrated the possibility that low molecular weight chelators (like chelating pollutants) can catalyze the release of iron from its siderophore complex and thereby influence its bioavailability.

Studies are being conducted to determine how a number of natural and synthetic drugs may show antibiotic activity because of their ability to increase oxygen toxicity in microorganisms. The toxic effects of oxygen are handled in virtually all aerobic organisms by an enzyme called superoxide dismutase (SOD). An anomalous SOD has been found in Photobacter leiognathi, a bacterium which lives symbiotically with the Pony Fish. This anomalous SOD occurs, most probably, as a result of a gene transfer from the fish to the bacterium. This is the first documented instance of natural gene transfer from a eucaryote to a procaryote. The question of why this anomalous SOD gene has persisted in P. leiognathi may relate to some of the studies being done by others. The anomalous bacterial SOD is one which has copper and zinc (in contrast to iron and manganese). It appears as though the conditions under which the bacterium must live within the Pony Fish is one of iron deficiency. This iron deficiency, imposed by the host, is actually one of the host's defense mechanisms against bacterial infection. Hence, for a bacterial symbiont, getting sufficient iron would be a chronic problem; and acquisition of a copper-zinc SOD by gene transfer would relieve part of its iron need. Tests are planned to see if induction of copper-zinc SOD is facilitated by iron deficiency, and alternatively, non-facilitated by iron-rich media. These studies may have far-reaching implications with respect to metal induced mutation, gene transfer, etc. The putative gene transfer,

first shown in P. leiognathi and the Pony Fish, is probably not a unique event. Now that scientists have been alerted to the possibility, other instances will most certainly soon come to light. There is already a report of an anomalous iron SOD in certain types of plants, and gene transfer may explain this anomaly. The phenomenon of gene transfer and the influences of environmental conditions on that phenomenon may have contributed importantly to biological evolution.

Red blood cells of marine and terrestrial species are being analyzed as possible targets for pollutants. Continuing studies show that hemoglobin functionality is altered by lead. Studies carried out with isolated hemoglobin in vitro show distinct changes in hemoglobin oxygen affinity. In spite of changes observed in vitro, blood samples obtained from lead-poisoned individuals have not shown significant alteration in oxygen-binding properties.

Structure-function experiments have continued along the lines of using hemoglobin from man and a variety of other species as a model protein system. It is perhaps noteworthy to report that a major pharmaceutical laboratory has chosen human hemoglobin as a model system for the development of pharmaceutical chemicals. Electron-transporting proteins are also being worked on in these laboratories. Studies on cytochrome c and cytochrome c oxidase have been carried out. The latter enzyme, a membrane-bound protein found in mitochrondria, has been shown to be strongly inhibited by various forms of mercury.

Studies are being conducted on the interactions of heavy metal ions with a variety of marine organisms. The interaction of heavy metal ions with the oxygen-carrying hemocyanin molecules of blue crabs, Callinectes sapidus, has been examined. In addition, experiments were carried out with hemolymph of blue crabs which had been exposed to cadmium. These studies have made it possible to analyze and understand how heavy metals interact with this hemocyanin in vitro and how this interaction produces profound changes in structural and functional properties of this oligomeric respiratory protein. Addition of heavy metals such as zinc, copper, cadmium and mercury induces a tremendous self-association of the hemocyanin molecule. This is completely reversible upon removal of the heavy metal. The association was also observed to occur in hemocyanins of other marine organisms. In addition, the heavy metals were also observed to alter the oxygen-binding properties of the hemocyanins.

Progress is being made in the crystallization of a 50,000 dalton domain of a molluscan hemocyanin. A 5-angstrom model of the hemoglobin of a marine fish, Leiostomus xanthurus, has been prepared. Significant progress has also been made in determining the structure of sulphite oxidase.

Studies have continued of structure-function relationships in metalloenzymes, particularly those containing molybdenum. Previous emphasis has been on enzymes such as sulphite oxidase, xanthine oxidase, nitrate reductase, formate dehydrogenase, aldehyde oxidase and nitrogenase.

A recent discovery has focused attention specifically on the metal molybdenum. All of the above-mentioned molybdenum proteins possess molybdenum in a unique form--tentatively called molybdopterin. Molybdenum in this form may in fact be one of the newest vitamins to be discovered.

Both developmental and behavioral studies have shown quantifiable alterations when marine organisms are exposed to extremely low levels of toxic metals,

pesticides and insect-juvenile-hormone mimics (which are used by the agrichemical industries to control insects). The effects of muds obtained from off-shore oil drilling rigs on the development of a variety of marine organisms are being studied as are the effects of a number of "agrichemicals" on developing crab larvae. These studies have shown that extremely low concentrations of agrichemicals have profound effects on the development of crabs as well as other marine organisms. Synergistic effects with the chemicals have been observed and are probably important in the real-world situation.

Studies on the transport of mercury across membranes have continued. An important finding has been that the uncharged form of metal pollutants, i.e., $HgCl_2$ vs. Hg^{2+}, is the form of the metal which has extremely high permeability through membranes. These transport studies have been extended to transport of various acid species across membranes. In the case of nitric and sulfuric acid (and probably other acids which occur in Acid Rain), it is the uncharged chemical species which has high permeability through membranes.

Studies have begun on the effects of heavy metals on the morphology and physiology of the teleost urinary bladder. Early studies of teleost renal tubules and amphibian urinary bladders have suggested that permeability and ion-transport characteristics change in these membranes in response to exposure to cadmium, platinum and organic mercurials. Additionally, platinum-containing antineoplastic drugs alter both the osmotic water-flow response and the cell surface response to vasopressin in granular cells. The platinum complex was also demonstrated, by x-ray microanalysis, to localize in the nuclear membrane of the granular cells. The objectives of these studies, which have just recently begun, are to determine the morphological responses of osmoregulatory cells of the teleost urinary bladder to various heavy metals and to evaluate the relationship between the observed structural changes and the functional alterations induced by the heavy metals.

The effects of pressure and anesthetics on central synaptic transmission are being examined. Work is being done to employ state-of-the-art techniques of physiology to study the biological effects of pressure and to use the tool of pressure as a thermodynamic variable in biophysical research. A high-pressure chamber has been built which allows for stable microelectrode recordings from a variety of intracellular preparations at pressures of 100 to 200 Atmospheres. Its features include temperature control, internal visibility, continuous profusion, electrical penetration and the manipulation of several internal controls, all being possible while the system is at pressure. This device is being used to study volatile compounds and inert gasses which must be raised to pressure higher than ambient in order to exert their effects.

A new laboratory which concerns itself with "immobilized species" has been set up. This laboratory, the Protein Engineering and Technology (PET) laboratory has been established to investigate the use of immobilized proteins and enzymes. The laboratory has been set up with the goal of understanding and developing immobilized proteins and enzymes. Hemoglobin, for example, has been immobilized on a number of different supports and then characterized. A significant advance has been the development of novel methods which allow high concentrations of proteins to be immobilized. These methods open a number of possibilities, among which are systems which can be used to filter water, air, or even blood.

University of Southern California Marine Biomedical Center

Research programs were conducted in the areas of (1) systems modelling, (2) transportation of societal waste, and (3) biomedical problems. A core unit covering general research activities was established.

Several professionals were listed in the original MFBC grant application as being available to work on the MFBC projects during the period requested. Some did not participate for various reasons, and others had various levels of participation. Only a few scientists received direct financial support.

Over the three years changes in emphasis developed. A number of foci have appeared. The first relates to the problems of the toxicity of pollutants in the coastal waters. Studies have been made of the fate of organic and inorganic pollutants. Analysis of heavy metals in tissues from bivalves and sea urchins demonstrated their presence in high molecular weight fractions. The research has been extended to show that chelation occurs with metallothionein in a series of tissues up to a limit beyond which tissue damage occurs.

Bioassay methods have been developed for evaluating pollutants present in sea water using fertility of sea urchins as the test object. A method was also developed for maintaining peritoneal macrophages from fish in culture and for cellular immunity.

A continuing aspect of our program has been the West Coast Squid Research Center. The squid giant axon, the giant neurons of the stellate ganglion, the neuronal assemblages of Aplysia and many nudibranchs provide excellent material for basic neurobiology and pharmacological and toxicological assessment of environmental pollutants. It was found that squid can be captured in adequate numbers for research from October to March. An organized collection and delivery program has proceeded in two locations. At UC Irvine holding tanks were constructed and studies were continued on the mode of action of anesthetics on the membrane of squid giant axon.

The area of marine bioactive substances is also being explored. The studies of the past two years have included extracts from selected sponges and tunicates. Promising activities have been obtained of extracts showing pre-synaptic release of neurotransmitters and potent repellent activity against sharks.

Studies on the problem of paralytic shellfish poisoning in California have continued. The intoxification of clams by ingesting cells of the dinoflagellate Gonyaulax catenella has long been known in cold northern waters. Incidents and fatalities are now occurring with increasing regularity in warmer, more southern waters on the east coast and in southern California. Research on the metabolism of dinoflagellates by these groups have shown that dinoflagellates can incorporate and metabolize organic compounds from the water. Modelling studies have begun on the transport process of PSP by the interaction of coastal current, meteorological and nutrient waste parameters on growth potential.

It has been found that fish living in the polluted environment develop vascular damage similar to that resulting in mammals from stress. The results can be reproduced by feeding the fish cholesterol.

Fish cells have been shown to respond to a variety of mutagens and carcinogens in a manner similar to that observed in mammalian systems. The difference, however, lies in the tremendous variability in these responses exhibited by normal cells from different fish species. Some of the striking differences recorded thus far include: (1) capacity of cells from a single species to grow and function normally over a temperature range in excess of 15°C (15-33°C); (2) absence of extremely low activity of mixed function oxygenase (MFO) activity in some species and highly active MFO systems in others; (3) the absence of an HGPRT enzyme system (or insensitivity to the analogues azaguanine and thioguanine); (4) the capacity of some cell lines to continue proliferating in the presence of relatively high concentrations of genotoxic agents even when extensive chromosomal damage is evident.

Two fish cell lines were examined for their capacity to produce forward mutations following exposure to known mutagens. BF (bluegill) and RTG (trout) cells were tested for their ability to metabolize promutagens and to produce clones under dilute plating conditions. The trout cells readily metabolized benzo(a)pyrene (B(a)P) to water soluble phenolic compounds but did not clone. The bluegill cells produced clones but only poorly metabolized B(a)P. Due to the necessity to produce clones in order to detect mutations in vitro, the BF cell was used for mutagenesis experiments. When exposed to the alkylating agent MNNG, the frequency of mutant cells, which were resistant to the ATPase inhibitor ouabain (OUA), increased. Exposure of this cell line to B(a)P resulted in a significant, but much less dramatic, increase in mutant frequency. Prolonged growth of the cells in non-selective medium did not diminish their level of resistance to OUA.

Because of the high MFO activity observed in the RTG cell line, it was chosen as a cytogenetic model for detection of chromosomal macrolesions. The sister chromatid exchange (SCE) technique has been demonstrated to work in fish systems but there are some major drawbacks to its use in all species of fish. Most fish species have exceedingly small and numerous chromosomes, making resolution of SCE's far more difficult than in mammalian systems. For this reason, only a limited number of species are suitable for use with this technique. In order to overcome this problem of resolution, a series of abnormal events during anaphase which can lead to the production of micronuclei or outright cell death were examined. The frequency and severity of anaphase chromosome damage, or alteration, were found to be proportional to the concentration and class of mutagen/carcinogen to which the cultured RTG cell is exposed. Five classes of known mutagen/carcinogen were all active in this system and produced frequencies of abnormal anaphase figures in excess of 90% in some cases. Exposure of the cells to several related chemicals with no known mutagenic/carcinogenic activity resulted in no increase of abnormal anaphases over that observed in untreated or solvent treated controls.

Toxaphene is a hepatic carcinogen in rodents and an aquatic pollutant which bio-accumulates and alters growth, bone development and reproductive success in fish. Toxaphene-exposed fish exhibit scoliosis, lordosis, and fragile backbone, with decreased collagen and vitamin C and increased mineralization in the vertebrae. Studies are being conducted to determine the consequences of toxaphene exposure to trout cells.

Untreated and DMSO-treated controls did not differ. At 15 µg/ml growth was reduced by 50% and many fibroblasts contained cytoplasmic vacuoles and crystalline inclusions. Decreased gylocogen and increased cytoplasmic density with concomitant

basophilia of hepatic parenchymal tissue were apparent by light microscopy in trout exposed to 200 ng/l toxaphene. Ultrastructural changes included an increase in rough endoplasmic reticulum and number of mitocondria, proliferation and misplacement of bile canaliculi, and a reduced space of Disse.

The in vitro 50% growth inhibition value of 15 ug/ml (15ppm) for toxaphene-exposed cells was considerably higher than the in vivo LC50 value of 10.6 µg/l (10.6 ppb) for a 96-hour assay, indicating that RTG-2 cells are less sensitive to toxaphene than rainbow trout fry. Although RTG-2 cells possess some mixed function oxidase activity, they may not be converting toxaphene to its most cytotoxic form, i.e., heptachlorobornane.

Various carcinogens (e.g. aflatoxin) induce hepatic neoplasms in rainbow trout. At least two basic types of hepatocellular carcinomas have been described in the trout, i.e, the trabecular type, which consists of broad traceculae of basophilic cells with moderately to greatly enlarged hyperchromatic nuclei, and the hepatocholangiolar or mixed carcinoma, which contains both trabeculae and centrally located biliary ducts surrounded by a connective tissue stroma. Cholangiomas also have been induced in fish by the carcinogens diethylnitrosamine and methylazoxymethanol acetate. The observed alterations in livers of toxaphene-exposed trout, especially the presence of aberrant bile canaliculi, may represent preneoplastic stages and warrant further study with a larger sample size.

Research has been undertaken to investigate the influence of thyroid hormones on adult hemoglobin expression in smolting coho salmon. Preliminary findings indicate that the timing and sequence of appearance of adult hemoglobin fractions in temperature accelerated zero-age smolts are essentially the same as those reported for yearling smolts. This appears to rule out absolute age as a criterion for adult hemoglobin expression. Currently the question of thyroidal involvement in this process is being addressed by means of administration of thyroid hormones and/or goitrogens in the diet or coho salmon, in order to create chronic elevation on depression of plasma thyroid hormone levels in treated fish. Plasma thyroid hormone levels are being evaluated by means of radioimmunoassay. The degree of adult hemoglobin expression in treated fish is simultaneously being assessed by integration of densitometric tracings of hemoglobin electrophoretograms, and by direct spectrophotometric determination of the concentration of individual hemoglobin fractions after elution from the electrophoresis medium.

AIR POLLUTANTS AND RESPIRATORY DISEASE

Work has resulted in the development of the guinea pig model for immediate onset pulmonary hypersensitivity to inhaled chemicals. The model simulates the industrial situation by employing inhalation as the root of exposure and excluding the use of adjuvants for enhanced immunologic stimulation. Toluene diisocyanate (TDI), a major component in the formation of polyurethanes, and well documented as causing industrial pulmonary sensitivity, was selected for use in developing the animal model. Groups of guinea pigs exposed to various concentrations of TDI over a three-hour per day five-day period of time indicated the existence of both a dose response relationship and a threshold level for inhalation sensitization to TDI. The study implies that an exposure concentration for TDI can be recommended for industry which would prevent sensitization of workers. In this connection, a study was completed which demonstrated that exposure of 103 workers for three years to 0.02 ppm TDI did not result in any cases of pulmonary TDI sensitivity.

Recent results demonstrate that during high frequency (10 to 20 Hz) ventilation (HFV), efficient gas transport can take place even with tidal volumes less than the dead space. Aerosol transport into lungs during HFV was examined to determine if the particles would penetrate into the lungs beyond the dead space. The findings were that HFV transports aerosols, as well as gases, between the tracheal outlet and the lung periphery. Substantial aerosol deposition occurs in central airways, but aerosol is seen even in very small airways and distal lung structures. The extremely low-particle diffusivity suggests that convective mechanisms, not diffusion, are primarily responsible for the transport. Aerosol is transported at speeds comparable to oxygen in branch tubes but slower in a smooth pipe. This suggests that asymmetries across bifurcations, perhaps in velocity profiles, contribute to the transport. Compared to normal ventilation, HFV results in a 3.5 fold increase in the ratio of airway to parenchymal deposition.

Studies are underway to determine if changes in pulmonary resistance and/or airway reactivity induced by pollutant exposure are related to changes in airway permeability in both normal and allergic sheep. Studies with ozone indicate that ozone alters airway permeability, that it also alters airway smooth muscle responsiveness, that both mechanisms play a role in pollutant induced non-specific airway hyperactivity and that O_3 promotes the release of endogenous histamine which may present a problem in allergic patients because of these already hyperactive airways. It is also shown that ozone can enhance the severity of bacterial pneumonia when the bacteria are already present in the lung. Together these findings suggest that O_3 does produce alterations in normal airway function and can pose a risk to already-compromised individuals.

Studies involving sensitizers have shown that various compounds, including dyes, compounds of biological importance like chlorophyllin and hematoporphyrin, and environmental pollutants such as polycyclic aromatic hydrocarbons (PAH), can sensitize the formation of singlet molecular oxygen at atmospheric pressure. The discovery that PAH and oxygenated PAH can sensitize the formation of

singlet oxygen is also environmentally significant. These compounds were found on the surface of particulate matter which may be in the respirable range. This particulate matter is in the atmosphere at a significant time, and mechanisms for the oxidation of particle bound organics to mutagenic materials could involve singlet oxygen. In short, PAH could sensitize the formation of singlet oxygen at the surface of the particle and in turn be oxidized forming in some cases mutagens. The current study has implicated singlet oxygen as a low-level atmospheric oxidant. Present experiments suggest that singlet oxygen is responsible for inflammatory processes of the lung, either by acting directly or by producing oxygenated materials in the atmosphere that are known to cause deleterious health effects.

A study was done of random samples of adults and children in six cities with different concentrations of air pollutants. Concentrations of pollutants have been measured outdoors and indoors as well as some concentrations by the use of personal monitors. A continued analysis of the possible effects of NO_2 from gas cooking has been carried out. Repeated analysis with additional cohorts and with a more appropriate adjustment for social class has not confirmed the original observation of an effect on FEV_1 or on illness before age two. Maternal smoking was shown to be associated with a small but significant reduction in FEV_1 and an increase in most respiratory symptoms most of which were statistically significant in children. Paternal smoking had no such an effect. The analysis of the children in the six cities on a cross-sectional basis showed a small increase of some respiratory disease associated with the increase in pollution. The respiratory diseases were a history of bronchitis in the past year, persistent cough in the past year, and lower respiratory illness index. Validation of these findings will be carried out. Another aspect of the study involves the analysis of the elemental composition of course and fine particles of over 2700 sampling days in an effort to quantify source contributions in each of the cities. Using factors and regression analysis techniques, several major sources in each community have been clearly identified. These include automobiles, residual oil, soils, coal, and transported sulphur, along with others. It was shown that sulphur as $(NH_4)_2SO_4$ contributes between 35 and 55% of the fine particle mass on average in different cities. The results of neutron-activated samples of indoor/outdoor particles collected in two cities are beginning to quantify the penetration factor for outdoor aerosols. Using certain metal tags for outdoor air, the current best estimate is that 40-70% of the ambient aerosol is penetrating indoors.

The biochemical effects of air pollutant oxidants are being investigated. Studies on the reaction of ozone have demonstrated that high protein concentration and low ozone concentration yields a specific oxidation of tryptophan. Under more rigorous conditions such as high ozone concentrations in an acidic environment, the oxidation is less specific. The oxidation of methionine does not play a role in enzyme inactivation. The oxidation, however, does result in a change in the tertiary structure of the protein. Other studies on the reactions of ozone with slow reacting sulfhydryls on tryptophan do not participate in inactivation of enzymic activity. It has also been found that lipid synthesis in macrophage was little affected by ozone, whereas in hepatocytes, this was the most susceptible activity.

PHYSICAL FACTORS

Assessment was made of the stimulus properties of intense 918- and 2450- MHz microwave fields with respect to mice and rats. Marked species differences were observed in which rats exhibited 100% mortality and mice zero mortality during uncued exposures in a continuously available behaviorally contingent 2450-MHz field. It appears that mammals larger than a mouse, because of smaller surface area to body mass ratios, which are correlated with reduced rates of dissipation of thermalized energy, have a difficult time perceiving time of onset and cessation of an intense field and may even fail to attribute delayed sensations of warming to an external source. These findings have implications for human workers in industrial environments in which very highly powered ratio frequency sealers and dryers are used.

Previous reports have demonstrated that microwave irradiation increases blood brain barrier permeability in mice. Additional experiments to substantiate this finding have been carried out and proved to be positive. In further studies using a higher power density and longer exposure time in phenobarbital anesthetized rats, biliary tree permeability changes were assessed by retrograde intrabiliary injection of radioactive sucrose and mannitol. Since recovery of these two marker compounds in recollected bile changed in opposite directions, it could not be definitely established that a permeability change occurred. But after once a day irradiation for four days, recovery of both marker compounds increased. Since these changes could not be ascribed to changes in bile flow, it appeared that a decrease in biliary tree permeability had been produced.

Rats were exposed to 2.45 GHz microwaves four hours daily up to ten consecutive exposures. Specific absorption rate was 0.20 W/kg/mW/cm^2 in 342 g rats. Effects of microwave exposure were increased colonic temperature, increased corticosterone level, decreased thyrotropin and growth hormone levels, loss in body mass during exposure and decreased weight gain. All changes noted were transient upon removal from the microwave field. Loss in mass during exposure and short-term weight gain were reversible during repeated exposures. Colonic temperature remained elevated although of decreased magnitude after 5 exposures at 40 mW/cm^2. Adrenocortical acclimation to microwave exposure was noted by the tenth exposure.

Two Chinese hamster ovary cell mutants sensitive to gamma irradiation have been isolated using nylon cloth replica plating and a dark field photography approach. One cell was found to have significant amounts of gamma-ray sensitivity and was selected for further study. This cell has a two-slope survival curve and an initial steep slope and then a flattening of the curve at about 10% survival, suggesting that the cell population may be composed of a resistant and more sensitive fraction. Sensitivity of this cell through the cell cycle was examined by irradiating populations of cells at various times after initial synchrony in mitosis. This cell is sensitive to gamma irradiation during G-1 early S phase and late G-2 periods of the cell cycle. During the persistent period (S phase), the survival to gamma irradiation approaches that of the parental cell. Cytological analysis of chromosome breaks indicate that the mutant cell has a much higher frequency of chromosome breaks at a given dose of gamma rays than does the parent cell. Also, this cell's sensitivity seems to be specific to gamma irradiation. This appears to be the first gamma sensitive mutant isolated in the tissue culture system. To date the only other source of these classes of mutants are from patients with the disease ataxia telangiectasia.

There are inconsistencies in the reported effects of microwave radiation on neural systems in vitro which may be attributed to experimental difficulties encountered in this type of research. In order to avoid or minimize some of the problems, an investigation of the effects of microwave exposure on freshwater alga Chara corallina was carried out. Mature single internodal cells of this alga have bioelectrical characteristics similar in many respects to mammalian nerve axons and other excitable tissues. It is thus possible to apply well-known neuroelectrophysiological techniques and methods of analysis to investigate the effects of microwave exposure on this excitable cell. The cells were exposed to CW, pulse-modulated and sinusoidally-modulated S-band microwave fields in a temperature controlled wave guide exposure chamber. The dependent variables measured before, during and after exposure to the S-band microwave fields included resting potential, amplitude of the action potential, rise and decay time of the action potential, conduction velocity and excitability. The Chara corallina cell population maintained at $22^{o}\pm0.1^{o}$C. during exposure showed no consistent or statistically significant microwave dependent alterations in any of the dependent variables.

BIOLOGICAL AGENTS

METALS

Studies on the pathogenesis of lead encephalopathy have demonstrated that daily doses of lead acetate, which are required to produce a cerebellar encephalopathy by behavioral and light microscopic criteria in at least 50% of rat pups, increase in animals begun on lead feedings at progressively older ages from 14-18 days. Pups fed lead from 20 days show only a patchy edema by light microscopy while pups fed from 24 days have no cerebellar pathology by light microscopy. The cerebellar and cerebral fine structure were studied by transmission electron microscopy in younger lead-sensitive animals and older lead-resistant animals. By EM, there were small areas of edema and neuronal necrosis in the cerebrums of the younger animals and in the cerebellums and cerebrums of older resistant animals. In all of these less-affected regions, there are lead-containing densities in nuclei, lysosomes, and cytoplasm predominantly or solely of astrocytes. This provided evidence to support the high hypothesis that the brain becomes resistant to lead toxicity as the capacity to sequester lead in non-critical cellular sites develops. In addition, in vitro studies of the effects of lead on primary cultured astrocytes were carried out. These studies demonstrate that the astrocyte in primary culture shows two characteristics in situ resistance to lead toxicity and ability to sequester lead in nuclei and lysosomes. A Na^{+}-dependent respiratory component in primary cultured cerebral astrocytes in media of increased osmolarity was demonstrated. This respiratory component is not coupled to oxidative phosphorylation or to a Na^{+}-K^{+} ATPase but appears to be coupled to a mechanism for excluding Na^{+} from the cell. Inhibition of this respiratory component in media of decreased Na^{+} concentration may be an important mechanism in the swelling of astrocytes with decreased osmolarity. Thus the respiratory property may represent an energy-coupled mechanism important in the swelling of astrocytes in conditions resulting in brain edema, including lead encephalopathy.

Preliminary findings in a study indicating that lead is relatively more inhibitory to rat brain ($Na^{+}K^{+}$)-ATPase were essentially duplicated using another series of brain preparations. The K^{+}-pNPPase activity remaining at final lead concentrations of 5 uM was 17%, 40%, 57%, and 74% of control at 5, 10, 20, and

60 days of age, respectively. At a final lead concentration of 10 uM, the values were 10%, 20%, 42% and 56% respectively. These concentrations are in the range of lead concentrations found in lead-intoxicated children--5 uM lead is equivalent to about 100 ug/dl. These findings may explain some of the susceptibility of immature brain to the cerebral edema and other signs of lead poisoning. Final work on effects of $(Na^+ + K^+)$-ATPase and K^+-pNPPase activities after exposure of rat cerebral microsomes to conditions of mild periodate oxidation was completed. Half-maximal inhibition was obtained at only 3 uM concentrations of periodate under these conditions. It is conceivable that in vivo oxidants may play a role in physiological and pathological states in controlling the activity of $(Na^+ + K^+)$-ATPase.

Recent studies suggest that lipid peroxidation due to various chemicals is directly responsible for loss of hepatocyte viability. A study of this phenomena suggests that lipid peroxidation is not totally responsible for the loss of cellular viability associated with incubation in different mediums or in response to chemicals such as sodium iodacetamide and diethyl maleate. In a related study, isolated hepatocytes were employed to examine the role of lipid peroxidation in copper toxicity. The results suggest that lipid peroxidation is not the cause of the injurious effects of this metal in isolated rat hepatocytes. In another study the rate of removal of cadmium from the blood indicated that three hours after injection, the concentration of cadmium had decreased almost two orders of magnitude and then remained relatively constant for twelve hours before increasing by sixty hours. To determine the mechanism for the decrease in blood cadmium concentration with time, the distribution of cadmium among the blood components was examined at five and 60 hours after administration. The concentration of cadmium decreased during this time interval by 95% in the plasma, did not change in white blood cells, and was increased approximately two fold in red blood cells. The concentration of cadmium in the ghosts and cytosol of the red blood cells increased to the same degree. Examination of the binding of cadmium to various red blood cell cytosolic proteins revealed that cadmium was bound mainly to high molecular weight proteins at both five and 60 hours after cadmium administration. At 60 hours, the second protein peak with bound cadmium was observed which had a molecular weight similar to metallothionein but differed from it in other characteristics. The results suggest that redistribution of cadmium to blood is due to the increase and concentration of cadmium in the red blood cells. This increase does not appear to be solely due to an increase in metal-binding proteins in the cytosol of red blood cells.

Electrophysiological and morphological measures are being used to assess the effects of low-level perinatal exposure in the rat on neurological development. The effects of postnatal, preweaning lead exposure on morphometric alterations in the hippocampal formation of the 15-day old, the 90-day old, and the 600-day old rat were determined. Rat pups were exposed to lead via the milk of dams drinking 0.2% lead acetate beginning at parturition. Mid-dorsal sections of the hippocampal formation were examined by light and electron microscopy in the preweaning, adult and aging rat. The findings were that lead exposure reduced neuropil development as evidenced by reduced areas of the dentate hilus and dentate infrapyramidal stratum moleculare and by increased numbers of hilar CA3 pyramidal cells per unit area. In addition, lead exposure reduced the numbers per unit area of several types of synaptic profiles in selected regions of the CA3 region of the hippocampus. The studies revealed that lead exposure during the postnatal period preferentially affects later-developing structures within

the hippocampal formation, rather than affecting structures already mature at the time of exposure. The limited exposure to relatively low levels of lead during the postnatal preweaning period produced subtle effects which persisted throughout the life of the animal, or at least until day 600. The principal effect of the lead exposure at each of the three ages studied, 15, 90 and 600 days of age, was on the large complex invaginated synaptic profiles of the mossy fibers which terminate in region CA3 of the hippocampus. Although the morphometric studies do not deal with the functioning of this system, they do reveal that structural aspects of these fibers are sensitive to the effects of lead during the developmental period and that these effects persist even into senescence. Since motor impairments and seizures are frequent neurologic sequelae of excess lead exposure in children, the relative significance of such symptoms in an animal model were evaluated. The results suggest that the lead exposure levels used were at or near a no-effect level for the several common neurobehavioral paths and other indices that were used to evaluate the effects. It appeared that kidney weight may be a more discriminative index of excess lead exposure than some simple neurobehavioral indices.

One investigation is concentrated upon the effects of cadmium which has been shown to have both immunoenhancing and immunosuppresive effects, depending on the cadmium concentration. In lymphocyte cultures, low concentrations of cadmium are stimulatory and high concentrations inhibit lymphocyte transformation. The effects of cadmium on the immune system were compared in vivo and in vitro in several systems. In addition to providing a basis for comparison, these studies are significant in that they demonstrate an effect of cadmium at levels approximating and including that currently permissible in drinking water in the United States (0.01 ppm).

A study is being done to determine the cellular and molecular pathogenic mechanisms whereby continuous low-dose congenital exposure to methylmercury results in smaller than normal offspring and the effects of these changes on postnatal growth and behavioral development. In order to characterize the effects of heavy metals on morphogenic processes operative early in development, mice were dosed with sodium arsenate on days 7, 8, or 9 of pregnancy and the results compared to fetuses of saline-treated dams. Within 7 hours of arsenic treatment, ultrastructural alterations in neuroepithelial cells were observed. These consisted of dense-staining inclusions thought to be autophagic vacuoles and small, apparently empty vesicles. Mesodermal cells were affected except in severely affected fetuses. Results indicate that necrosis in the fusion zone in the neuroepithelial transition zone (that region of the neural fold designed to meet the opposite folds and fuse) may explain nonclosure of the neural tube in the head region.

The effects of in vivo cadmium and lead exposure on human lymphocyte transformation was investigated using peripheral blood mononuclear cells from normal volunteers. Cadmium and lead, as their chloride salts, were used at concentrations of 0.1-1000 uM and their effects on unstimulated and Con A stimulated DNA synthesis measured during in vitro exposure. In both unstimulated and Con A stimulated lymphocyte cultures, Cd^{2+} at 100 and 1000 uM was associated with the total inhibition of DNA, RNA, and protein synthesis. At 0.1-10 uM, Cd^{2+} was slightly inhibitory for macromolecular synthesis except in unstimulated cultures where DNA synthesis was enhanced. Lead increased DNA synthesis and slightly inhibited RNA and protein synthesis in unstimulated lymphocyte cultures and had no effect on Con A-stimulated cultures except at 1000 uM where DNA, RNA and protein synthesis were all inhibited.

The sites of action and the delineation of the neural mechanisms responsible for the decreased visual capacities observed in the scotopic receptor system following lead exposure during development is under study. Results demonstrate a subtle, long-term, visual acuity deficit in lead-exposed rats. Previous findings suggested that lead exposure alters retinal and/or cortical functioning. Both sites are known to contain muscarinic acetylcholine receptors. The concentrations of lead in zinc and various structures of the visual system and the brain are also being determined. It has been found that brain lead values for several different regions varied with 21 and 100 day exposures. These results are to be correlated with neurological effects.

Mercury detoxifying enzymes are being biochemically characterized. Mercuric reductase from E. coli containing the cloned mercury resistance genes from the plasmid NR1 have been successfully purified. It has been confirmed that mercuric reductase requires sulfhydryl reagents for activity. The substrate specificity of the purified enzyme was also determined to be quite high for mercuric ion. Spectrophotometric studies on this enzyme have been initiated. Attempts are also being made to detect mercuric reductase in the rat kidney.

Chelate antidotes for cadmium intoxication are under study. An attempt is being made to determine whether long-term administration of compounds which have been found to be capable of mobilizing cadmium from aged deposits in the kidney can lead to reduction of cadmium levels to values less than the "critical levels" and to determine whether such reductions are accompanied by at least a partial reversal in the histopathological and kidney function damage. The compounds which have the most pronounced effect appear to be those which yield cadmium complexes of a considerable degree of lipophilicity, which are appreciable lipophilic themselves or both. The amounts of cadmium which can be mobilized are a significant fraction of the total cadmium present when the most effective of these compounds are used. Most of the compounds which are able to mobilize cadmium from aged deposits appear to be ones in which certain types of sulphur donor atoms are present. Three compounds have been found which are capable of causing some depletion of the kidney stores of cadmium. Although the reduction in kidney cadmium load which was obtained was modest and because of the problems involved in raising the levels at which these compounds can be administered, it is promising to investigate the effect of lengthening the time during which animals receive these modest doses of the effective compounds. A series of such experiments are under way.

A better understanding of the molecular basis of the effects of methylmercury on cellular DNA and RNA synthesis is under investigation. It has been observed that methylmercury inhibits DNA and RNA synthesis in intact cells. Preliminary experiments demonstrated that in isolated nuclei methylmercury inhibits DNA synthesis but stimulates RNA synthesis. Additional experiments have confirmed that this is not an artifact. Experimental work has provided a possible explanation of these observations. It is believed that methylmercury inhibits initiation of RNA chains by all three polymerases (I, II, III) as well as elongation by polymerases I and III. In contrast, elongation by polymerase II is not inhibited by methylmercury and is even stimulated by it at some concentrations, since only chain elongation occurs in isolated nuclei. This result explains the effects of methylmercury on RNA synthesis in isolated nuclei and in whole cells.

The toxicity of manganese in the central nervous system is under investigation. The changes in brain amine content were measured after treatment of mice with

manganese chloride in their diet or by injections of the fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT). Both administrations produced similar changes in brain dopamine (DA) and GABA concentrations. Regional studies of DA and GABA concentrations have documented the area specific effects of manganese. Enzyme studies have suggested that cholinergic neurons, however, were unaffected by manganese. Follow-up studies are underway to determine the biochemical basis of this metal's effect and the pharmacokinetic disposition of manganese after acute exposures.

The mechanisms whereby exposure to nephrotoxic heavy metals leads to aminoaciduria is under investigation. Specifically, the effects of metals on amino acid carrier systems are being studied by measurement of the time required for amino acids to cross the tubular epithelium (epithelial passage time, EPT). Calculations, however, led to the conclusion that interaction between amino acids and their carriers in all likelihood contributes relatively little to EPT. Nevertheless, significant progress has been made in the study of the kinetic of metal inhibition of amino acid reabsorption. It was found that treatment with mercury reduces the maximum tubular capacity to reabsorb certain amino acids. In a homogeneous enzyme system, such an inhibition is described as being uncompetitive in nature. However, in a system as complicated as the renal tubule, this concept may not be applicable. Therefore, it is planned to study the effects of other nephrotoxic agents or of metals under conditions where an immediate inhibition of reabsorption can be observed. Thereby, the significance of the reduced saturation constants can be properly evaluated.

The effects of heavy metals on the vertebrate retina are being studied. Accurate and elaborate dose response curves for lead and cadmium, as those metals affect both rods and cones, are being established. These relationships tend to be somewhat complicated in that saturation and rebound phenomena appear to occur. In addition, it has been observed that high concentrations of these metals affect cone responses in the opposite directions to the rods. More data are being collected to help with this interpretation.

The inhibitory influence of selenium on hepatic microsomal drug metabolism in the rat is being assessed with particular regard toward the nature of the functional status of the biochemical components of this enzyme system. In addition, the protective effect of selenium against the toxic effects of cadmium on the system is being examined. These studies are of significance because it is known that selenium, although a toxic metal, is also an important micronutrient necessary for the maintenance of normal health. Therefore, it is important to develop more sensitive indicators than the grossly toxic effects of exposure to the metal. Preliminary studies have shown some differing effects on the interaction of the MFO systems in handling select chemicals.

The administration of organotin compounds in single doses in laboratory animals causes a significant and prolonged induction of heme oxygenase and a sustained decrease in hemoprotein content in the liver. Pure organotin produced a substantial and very prolonged induction of heme oxygenase accompanied by a steady decline in cytochrome P-450 content for periods up to eight days. The long duration of action of this organotin with respect to induction of heme oxygenase and depletion of cellular hemoprotein content provides a highly sensitive metabolic system with which to define further the toxic potential of organometals as well as to study the adaptive responses in liver to long-term perturbations of heme metabolism by foreign chemicals.

The absorption of toxics such as heavy metals is one of the most important factors in determining if an exposure will result in untoward health effects. It has been found that the mechanisms involved in cadmium and zinc transport in the jejunum are not competitive and, therefore, are not identical. Biosecretions strongly depressed jejunal transport of cadmium suggesting a role of bile salts. In addition, it has been found that absorption of cadmium by the small intestine of newborn rats exceeds that of adult rats. Mechanistic studies are underway to determine the basis for this difference.

The mechanisms whereby a variety of organisms differ in the way they use selenium and metabolize this element is being examined. There is a wide range of responses among organisms from highly sensitive to the toxicity of selenium to those organisms which require the element for their growth. Investigations on the utilization, transport and various chemical complexes of selenium are in progress to determine if these phenomena can be explained.

A study involving the efficacy of 29 compounds as antidotes for mercury poisoning has been carried out to determine the structural requirements for such antidotes. The studies indicate that agents containing two thiol groups on the same chelate molecule simultaneously bond to the same mercury species in a complex. From the data assembled on antidote effectiveness, it would appear that the presence of a second donor group is required, probably to provide the required kinetic stability for the complex. It has also been found that various antidotes will vary in their effectiveness when the toxic agent is inorganic mercury vs methylmercury chloride. The basis of these differences are being studied.

The chronic subtoxic effects of cadmium and lead ingestions on the cardiovascular system are being determined. Functional and metabolic changes in the cardiovascular system are being studied by utilizing in vivo techniques such as electrocardiographic, His bundle electrographic and cardiac contractility analysis as well as metabolite analyses to detect biologically significant pathological changes. Isolated perfused heart studies are also demonstrating effects of cadmium and lead exposures. Both biochemical and functional changes such as contractile tension velocity of contraction and tissue creatine phosphate levels were affected by these metals.

Mechanisms of lead interaction with nuclear proteins in nuclear inclusion bodies are under investigation. Clues are being sought as to whether inclusion bodies are a physiological or pathological response to exposure to these metals. Examination of proteins in the inclusion body fraction has identified a predominant protein which appears to be unique to lead induced nuclear inclusion bodies. The further characterization of this protein and the mechanisms of its formation in the nucleus will be of value in understanding how lead is dealt with in the nucleus of cells.

The metabolic effect of lead in humans is under analysis utilizing blood samples collected and screened from individuals who may have been exposed to lead in ambient or work environments. A detailed statistical analysis of the correlation between blood lead and erythrocyte protoporphyrin (EP) has been completed utilizing samples from children in New York City. The data indicate a threshold blood lead for EP elevation with an average estimate at 16.5 micrograms per dl. This value is near the mean for urban children and well below the 30 micrograms per dl presently considered normal. In addition, an epidemiological survey of childhood lead poisoning in a community surrounding a lead smelter in southern

Yugoslavia has found children under three years of age to be markedly affected by such exposures. Of those tested, a majority had blood lead concentrations between 50 and 69 micrograms per dl while 12% had 70 micrograms per dl or more.

The neurotoxicology of heavy metals is being studied using internal perfusion voltage clamp techniques for neuroblastoma cells. Since methylmercury is known to have potent blocking action on membrane ionic conduction mechanisms, the detailed mechanism of action of methylmercury on ionic conduction is being analyzed. Initial suggestions are that acute methylmercury poisoning irreversibly alters presynaptic function at the mammalian neuromuscular junction. This knowledge about the mechanism of action of methylmercury at the cellular and molecular level will help determine the site of action of the compound in nerve and muscle systems and hopefully help to identify how the compound reacts with ionic channels.

Brain capillaries are being isolated from control and lead-exposed calves in a study of the role of blood-brain-barrier in the development of lead encephalophy. Metabolic impairment of capillary function by lead is being assessed by measuring labelled glucose or glutamic acid substrates. The effect of lead on transport systems of brain microvessels are being determined by measuring isotopic chloride and calcium fluxes in endothelial cell suspensions of capillaries from control and lead-exposed calves. At diet levels of 10 mg/kg/day, a marked impairment of glucose catabolism has been revealed.

Preclinical studies of chelation therapy of childhood lead poisoning are underway. Lead poisoned rats have been treated with 2,3-dimercaptosuccinic acid (DMS) and the effects of this treatment on the metabolic function of brain, kidney, liver, and blood are in progress (animal studies). It is hoped that functional improvements of these organs will be obtainable by such treatment.

Research on the metabolism of lead is being carried out to determine how this material is handled in bone tissue. Morphological and biochemical interactions between lead and bone with an emphasis on metal-enzyme-hormonal relationships are under investigation using bone cells in culture. Clinical observations will be correlated with in vitro studies to define interactions between the vitamin D endocrine system and lead metabolism. Strong evidence has been presented that lead ion impairs the biogenesis of hormonal vitamin D by inhibiting the activity of the 1-hydroxylase enzyme in the mitochondria of renal cortical cells. This likely modifies the intestinal absorption of lead and the metabolism of endogenous lead.

BIOMECHANISMS

Studies were carried out to further characterize pyrimidine 5-nucleotidase deficient (PND) red cells in nonspherocytic hemolytic anemia. A study was performed on erythrocytes obtained on a patient confirmed as having PND using enzymatic and chromatographic techniques. Dilute suspensions of PND patient red cells were less filterable than normal cells using 3 micron pore-size filters. Anion exchange HPLC revealed an abnormally low ATP concentration accompanied by elevated CTP and UTP, as well as other pyrimidine nucleotides in PND patient red cells. These cells were also found to have a decrease in intracellular Mg-ATP and pH, despite an increase in magnesium concentration as determined by atomic absorption and ^{31}P-NMR spectroscopy. Red cell calcium was normal. These studies have further characterized both physical and metabolic defects associated with this disorder.

MUTAGENESIS-CARCINOGENESIS

This research project utilizes the epidermal cell lines derived from the hamster epidermal cell transformation assay. The parameters studied were several differentiation markers and the regulation of ornithine decarboxylase (ODC) after promoter treatment. It was found that epidermal cell lines derived from carcinogen-treated primary cultures fail to terminally differentiate as primary cultures do and that as they are passaged in culture, they exhibit a synergistic induction of ODC after 12-O-tetradecanoylphorbol-13-acetate (TPA) and fresh medium, similar to the response of preneoplastic and neoplastic hamster fibroblasts. Thus both preneoplastic fibroblasts and epithelial cells exhibit differences in their regulation of ODC activity which can be detected by promoter treatment. In addition, an analysis of the effect of TPA on "initiated" cells in the hamster embryo cell transformation assay were carried out. TPA can apparently rescue carcinogen-initiated but not normal cells from a program of senescence, thus greatly increasing the probability of these cells progressing to malignancy.

The objective of another study is to determine the effect of repair of alkylated germ cell DNA on mutation induction in Drosophila melanogaster. Studies indicate that there is a decrease in the ratio of tritium to carbon-14 from 13 in oocytes one to three days following treatment to 2 in oocytes four to six days following treatment of the repair competent Oregon-R stock with tribium labeled ethyl-methanesulfonate. In contrast, the stock homozygous for the excision repair-deficient mutant mei-9L1 was found to have a ratio of 29 and 22 respectively for days 1 to 3 and 4 to 6 respectively. A treatment of feeding Oregon-R females 4mM of EMS for 24 hours induced in oocytes a 0.8% relative frequency of sex-linked recessive lethals (SLRL), whereas a similar treatment to the mei-9L1 stock gave a yield of 6% SLRL in the brood one to three days following treatment and a 2% yield in the brood four to six days following treatment. These broods all represent treated oocytes. The increased rate of loss of tritium-labeled ethyl groups from DNA in the repair competent strain, Oregon-R, is in contrast to the slower rate of loss in the repair deficient strain, mei-9L1. This change in rate of loss is the first evidence that the repair deficient strain acts by blocking removal of alkyl groups from DNA in germ cells, as in previous experiments conducted with somatic cells.

A study is designed to develop a useful rapid bioassay for identifying environmental carcinogens in which the whole animal is used as the test system. The derived values for various environmental pollutants obtained upon the application of the analytical methodology is numerically related to those effects measured upon a localized exposure of the small bowel to ionizing radiation. This permits a comparison of the various environmental insults through utilization of a common denominator (localized X-irradiation of the hypoxic ileum and jejunum) thus permitting the development of a rational scale for the comparison of mutagenic/carcinogenic potential (Radiation Equivalency) of various environmental toxicants. Using this system it was found that one classic carcinogen, which the Ames Salmonella test fails to identify, carbon tetrachloride, was found to be positively identifiable in this system. The area of investigation was expanded to include organo-halogenated compounds which were found to induce antitumor cell-mediated immunity (CMI) including chloroform, dichloromethane, and dichloroethane. Further extension of this concept was a successful attempt to apply the measurements for identifying carcinogens in waste water. Urine was collected from carcinogen exposed rats and tested for the induction of antitumor immunity. Investigations of assay specificity were expanded, and the results

indicate that both the exposed animals' immune response (lymphoid cells) and the type of target cells utilized for the in vitro measurements determine the actual specificity of the analysis. By altering the target cells, carcinogens were identified having target organs other than the gastrointestinal tissue, in which case the assay was found to detect asbestos exposures, another classic failure of the Ames test. Basic immunological studies were carried out, and a common oncofetal protein was identified occurring in chemical (DMH-colon and 7, 12-dimethylbenz(a) anthracene-pancreas) induced cancer-bearing rats which was immunologically similar to that previously found in those animals having the X-ray induced small bowel adenocarcinomas.

The identification of several types of cytochrome P-450 and the considerable aromatic hydrocarbon hydroxylase activity present in microsomal preparations stands in marked contrast to the low activities generally observed when testing polycyclic hydrocarbons in the recessive lethal test in Drosophila. A possible explanation is that, with some procarcinogens the ultimate mutagen may not reach the target germ cells in doses sufficient for mutation induction. If this indeed is the case, somatic mutation assays may provide an alternative procedure for testing carcinogens in Drosophila. This possibility was tested in the system based on the determination of somatic recombination frequencies (twin spots) and mutations (single spots) in w^{co} sn^{2}/w; se h females, and of single spots in w^{co} sn^{2}; se h males. The results indicate that this assay can be helpful in evaluating the genetic effects of mutagens in Drosophila. Six carcinogens were assayed for the induction of somatic mutation and recombination in larvae, and all six gave a positive response. Particular advantage of this method may be seen in the very low frequency with which mitotic recombination and mutation occur spontaneously. The results show that somatic mutation tests may become a useful adjunct to the recessive lethal tests on germ cells.

A study is being carried out to understand the metabolic pathways and carcinogenicity of carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and carcinogenicity of carbonyl chloride ($COCl_2$). Although several in vitro studies have failed to demonstrate any significant binding of CCl_4, in vitro metabolic activation experiments under oxidative conditions using chromatin have not been done. Preliminary results indicate that metabolically-activated CCl_4 bound covalently to hepatic chromatin DNA; CCl_4 was also more reactive to hepatic chromatin DNA than exogenous calf thymus DNA. This covalent binding of CCl_4 to chromatin DNA needs further confirmation which will demonstrate for the first time that CCl_4 interacts to DNA and via an oxidative pathway. The results also show that CCl_4 binds covalently to chromatin protein and significantly more than it binds to DNA. Moreover, the extent of binding of CCl_4 was higher to non-histone chromosomal proteins than to histone proteins.

TEST DEVELOPMENT

One study relates to the utility of using skin oil (sebum) analysis as an indicator of body burden of chemicals derived from human environmental exposure. Studies were carried out in four groups of workers with exposure history to a variety of environmental contaminants. Analysis for the chemicals were made in fat, blood serum and skin oil. In roofers exposed to a variety of polycyclic aromatic hydrocarbons (PAH), PAH was determined in skin oil while none was detected in serum. In PCB exposed workers and PAH workers, skin oil analysis showed differences from the body burden as indicated by serum analysis. For

example, DDE in skin oil appears to be representative of the body burden but this is not clear for PCBs. It appears that the skin analysis for environmental chemicals may be a useful corollary to serum analysis.

ORGANIC CHEMICALS

Studies indicate that ortho-methyl substitution in nitrobiphenyls and nitro-naphthalenes usually inhibits mutagenicity, while an enhanced effect was observed in the corresponding amino-biphenyls and amino-naphthalenes. This result indicates that the steric or electronic factors required for N-oxidation or nitro reduction are different for both classes of compounds. Both 4-amino and 4-nitrobiphenyl are potent mutagens while the 3- and 2- isomers in both cases were inactive. This result suggests a possible formation of a common active intermediate from both nitro- and amino-biphenyls.

The objective of one project is to examine hepatotoxins that are not chlorinated hydrocarbons for inhibition of the calcium pump. Carbon disulfide was examined and was shown to inhibit the liver endoplasmic reticulum (ER) calcium pump. This suggests that inhibition of the liver endoplasmic reticulum calcium pump by hepatotoxins may not be an action observed only with chlorinated hydrocarbon hepatotoxins. Other chemical classes of hepatotoxins may disrupt calcium homeostasis by inhibiting the liver ER calcium pump. Another compound 1, 1-dichloroethylene (DCE) was of special interest because it does not produce demonstrable lipid peroxidation. When DCE is administered in vivo, it promptly produces damage to the ER. This finding is of special interest because electron microscopic studies from other laboratories have not demonstrated DCE-induced damage to the ER. In vitro studies with DCE have shown that calcium pump inhibition depends upon metabolic activation of DCE by the mixed function oxidase system and does not depend upon lipid peroxidation. Together these studies establish that DCE is activated by the liver to a toxic intermediate or intermediates that attack the ER calcium pump promptly after administration to an animal. Other studies have shown that CCl_4 specifically disrupts the ER contribution to calcium homeostasis. This work establishes a dose-response and a temporal relationship between the inhibition of the ER calcium pump and loss of calcium from ER membranes isolated in the microsome fraction. The work also demonstrates that these changes occur before there is evidence of damage to the plasma membrane. These findings are significant because they demonstrate the inhibition of the calcium pump and possibly release of an intracellular pool calcium occurs before there is external evidence of damage to the hepatocyte.

Nitropolynuclear aromatic hydrocarbons have been identified in the environment and are considered among the most potent mutagens in the S. typhimurium assay. 5-nitroacenaphthene (5-NA) and 1-nitronapthhalene (1-NN) were found by HPLC in airborne particulates and in diesel emission exhaust. 5-NA is carcinogenic in both rats and mice. In contrast, 1-NN showed no carcinogenic effect in both species under the same conditions. Using the S. typhimurium assay system, the mutagenic activity of 5-NA was much greater than that of 1-NN. Metabolism studies of both 5-NA and 1-NN were performed under the same conditions either aerobic or under 10% O_2 and N_2 atmosphere. Under aerobic conditions 5-NA gave metabolites produced from both nitro reduction and C-hydroxylations. 1-NN under identical conditions gave only metabolites produced from ring hydroxylations, and no nitro reduction was observed. The metabolism of 5-NA under 10% O_2 in N_2 gave mainly three major products. Under identical conditions 1-aminonaphthalene was detected in the metabolism of 1-NN. Mutagenic activities of representative

metabolites obtained from 5-NA were compared in S. typhimurium TA98 and TA100. The results of the mutagenicity assays indicate that 1-hydroxy-5-NA and 1-oxo-5-NA are proximate mutagens of 5-NA. The high mutagenic activity in bacterial systems and the carcinogenic activity in experimental animals, combined with the fact that humans are exposed even to minimal amounts of nitro compounds, demand more detailed in vivo studies to elucidate their mechanism of action.

A study was done to understand the biochemical molecular mechanisms by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds exert their toxic effects in mammals. Studies to date have shown that, despite large differences in species susceptibility to the toxicity of TCDD, these species do appear to have a TCDD binding protein with high affinity, low-capacity characteristics. In addition, relative affinities of other congeners of TCDD and compounds (3-methylcholanthrene, benzo(a)pyrene, B-napthoflavone) which induce the same class of P-450 associated monooxygenases as TCDD, are markedly similar. This appears to be unlike that observed in genetic strains of mice where great differences in number and/or affinity of TCDD for the receptor have been observed. With the exception of these strains of mice, other factors or properties of these receptors other than their relative presence may contribute to the relative differences in TCDD toxicity. These studies also suggest some relationship between the presence of receptors in the thymus and thymic atrophy induced in various species by TCDD. The studies also suggest some relationship between the relative affinity of these receptors for TCDD and the acute LD50 values for different mammalian species.

The gluthathione S-transferases comprise a group of multi-functional enzymes involved in the biotransformation/detoxification of a broad spectrum of hydrophobic compounds bearing an electrophilic center. Rat liver GSH-Trs have been reported to exist in multiple forms; however, there is a great deal of confusion regarding the number of isozymes and the selenium independent GSH peroxidase associated with them. Each isozyme of GSH-Trs from rat liver cytosol has been isolated and purified to homogeneity by employing a four-step purification procedure involving gel filtration, affinity chromotography, ion exchange chromatography and chromatofocusing. At least eight major isozymes, which include both anionic and cationic forms, have been identified and characterized by their physical chemical properties. Ligandin, an anionic binding protein, once reported to be associated with GSH-TrB has been distinctly separated from the latter. SDS gel electrophoresis of the mixture of purified GSH-Trs indicate that there are four different subunits.

A study was undertaken to establish that multiple P-450s result after phenobarbital administration. Furthermore, these P-450s are coded by specific mRNAs and are not the result of post-translational modification. It was previously shown that four immuno-chemically identical forms of phenobarbital-induced liver cytochrome P-450 exists in unique combinations which characterize different strains in colonies of rats. One colony of Long Evans rats only exhibited cytochromes P-450b_{LE} and P-450c among these immuno-related enzymes; whereas, one colony of Holtzman rats was characterized by cytochrome P-450b_H and P-450e. The results of these studies indicate that in vitro synthesized products exactly correspond to the two particular forms of the enzyme that characterize liver microsomes from each of these rat groups. It is concluded that different structural genes encode these immuno-related forms of cytochrome P-450 and that significant post-translational processing of their polypeptide products does not occur in vivo.

Studies on polybrominated biphenyls (PBBs) were extended to obtain more information concerning the effect of these compounds as inducers of drug metabolism and to evaluate the role of the change in drug metabolism on endogenous steroid metabolism. When pregnant rats received PBBs in their diet during gestation, there were some small neuroendocrinological changes observed in the pups. Body weight gain was reduced in both male and female rats exposed this way. The absolute weights of some organs, such as the ventral prostate and seminal vesicles in males and adrenal and pituitary glands in females, were reduced in animals maintained on the PBB diet. When these changes were expressed as percentage of body weight, only the ventral prostate weight remained significantly reduced. Exposure to PBBs did not affect plasma concentrations of lutenizing hormone, prolactin or corticosterone, nor did it affect the increase in the plasma concentration of the latter two hormones in response to stress. Exposure to PBBs did not alter the steady state concentrations of norepinephrine or dopamine in the posterior pituitary or selected brain regions. Furthermore, treatment with PBBs did not alter the rates of synthesis of these catecholamines in the brains of female rats on the day of diestrus or estrus on the basis of vaginal cytology. Exposure to PBBs significantly lengthened the estrus cycle of female rats.

The effect on catecholamines of carbon disulfide is being studied with the hypothesis that increased tyrosine hydroxylase (TH) activity in the fat of rats chronically exposed to carbon disulfide leads to an increase in tissue catecholamine concentrations and subsequently increases their turnover. This might lead to increased lipolysis and, therefore, the hyperlipidemia and subsequent vascular lipid deposition seen after chronic carbon disulfide exposures. It was found that increased TH activity in fat after chronic carbon disulfide exposure was responsible for the concomitant increase in catecholamines. Increased catecholamine synthesis through stimulated TH activity and increased catecholamine turnover may be responsible for increased blood lipid after chronic carbon disulfide exposure and may explain fatty deposition in the vasculature. The firm and persistent binding of CS_2 to hemoglobin which was observed provides new insights into the pathway of the solvent in the body. Although the significance of these findings is not clear at present, data such as these may be important in setting levels for continued chronic exposure to carbon disulfide and in the pharmacokinetic modeling.

The biochemical mechanisms underlying the neurotoxicity produced by chronic acrylamide exposures are being studied by evaluating the role of inhibition of the neuron-specific enolase (NSE) produced by acrylamide in relation to the development of the neuropathy. Initial experiments which showed an association between decreased NSE activity and neurotoxicity in acrylamide intoxicated rats were confirmed in cats. *In vitro* studies of acrylamide and interaction with enolase indicated that acrylamide produced a mixed type of inhibition which could not be overcome by the addition of excess substrate. Activity could be restored, however, by dialysis indicating that the interaction did not involve the formation of covalent bonds. When enolase was incubated with acrylamide and dithiothreithol, a sulfydryl protecting agent, activity could not be restored to control levels by dialysis. These results emphasize that energy production may be compromised by acrylamide as a possible first step in the development of neuropathy and that energy supplies in the axon would be impaired at two enzymatic sites. Furthermore, the specific inactivation of the neuronal isoenzyme of enolase may account for the selective toxicity of acrylamide to neurons, particularly the long and larger diameter axons which have the most demand for energy requirements.

Several toxicological studies were conducted to provide information to advance the understanding of the toxic actions af tetrachloroazobenzene (TCAB) and tetrachloroaxybenzene (TCAOB) (two important environmental and occupational toxicants) and the interrelationships of the mode of action of the isosteric compound TCDD. The toxicity of TCAB and TCAOB was examined in chick embryos via injection into the air cells of fertile eggs. The major malformation detected in the treated chick embryos was rump edema. Other abnormalities were detected in treated embryos but with lower frequency than this. These included altered feather pattern and lack of down, hemorrhage, external viscera, reduced body size, failure to withdraw from the yolk sac, beak/mouth malformation, dilation of blood vessels, and monomicropthalmia. Many of the toxic effects observed in lymphoid organs paralleled those seen in animals treated with TCDD. In addition, results obtained from mechanistic investigations are consistent with the hypothesis that the toxic actions of the chlorinated dibenzo-p-dioxins and the isosteric TCAB/TCAOB are mediated by the same molecular and biochemical/physiological mechanisms. Various pathological changes were observed in rats from a four-month feeding study with TCAB and TCAOB at 100 ppm concentrations. The most severe degenerative change is a massive vacuolization in the liver of TCAOB fed animals and, to a lesser extent, in TCAB fed animals. Other histological alterations similar to those seen in animals obtained from acute studies were also observed in lymphoid organs. Examination of lung sections revealed various inflammatory lesions in rats exposed to both TCAB and TCAOB. Pharmacokinetic profiles of TCAB and TCAOB in rats revealed rapid clearance of these compounds as opposed to strong binding affinity observed with TCDD.

INSECTICIDES

Published studies indicate that organophosphate insecticides can alter immunity in laboratory animals. The importance of such immunotoxic effects of these compounds on acquired immunity has not been adequately assessed relative to the demonstrated biochemical indices of organophosphate exposure and toxicity such as tissue cholinesterase and carboxylesterase activity. The plan is to treat laboratory animals with a series of organophosphate insecticides, then inoculate either with thymus dependent or independent immunogens to compare immunologic function. Initial findings suggest that toxic chemicals play an important role in the immunosuppression response. Some indication of selective action has been found and this further delineation of this response is being pursued.

The sites and mechanisms of organophosphate toxicity at discrete loci in the central nervous system is being examined. Organophosphates are known to act as potent inhibitors of acetylcholinesterase, the enzyme responsible for hydrolysis of acetylcholine and produce effects nearly equivalent to excessive cholinergic stimulation. However, when applied to the ventral medulla, organophosphates elicit a profound, long-lasting vasodepression which is reversed in the presence of the muscarinic antagonist atropine and oxine reactivators of the enzyme. These observations implicate the presence of neuroanatomically well-defined site on the ventral medulla and a mechanistic role for central cholinergic tracts in maintaining cardiovascular function. Studies are underway to identify these tracts employing fluorescent phosphonates. These fluors not only induce a vasodepressor response but also react with acetylcholinesterase to label distinct sites, hence rendering the neurons visible. The role of oxime reactivators in reversing the vasodepression and their capacity for distribution in the central nervous system will then be compared with the extent of enzyme reactivation, thus allowing estimation of their mechanism in bringing about the putative therapeutic benefit.

A biochemical-morphological model of methylmercury intoxication in cerebellar granular cells representative of central nervous system neurotoxicity is being sought. Protein synthesis in cerebellar granular cells is being characterized, structural and correlative biochemical studies of methylmercury interaction with the granular cells are being carried out, and molecular analysis of ribosome-mercurial interactions are being accomplished.

A recent awareness of the role of organometaloid compounds (e.g., the biomethylated species of mercury) has required the search for an elucidation of the early pathobiochemical insult in sensitive systems such as the central nervous system. Studies directed toward answering some of the questions relating to the molecular pathophysiology of methylmercury interactions are underway. So far, at least three sensitive sites of mercury interaction have been identified; a) the direct mitochondrial interaction with block of oxidative phosphorylation and reduction of high-energy phosphate, b) the demonstration of inhibition of cytosol factors needed for ribosome translation, and c) significant changes in potassium flux across cell and synaptosome membranes. Using isolated cerebellar cell preparations, combined biochemical and morphological studies of the system can be accomplished.

A project has been designed to assess the effects of prenatal exposure of mammalian embryos/fetuses to organophosphorus (OP) insecticides. The animal model chosen was the rat and the prototype OP was methylparathion, a compound which is used on a very large scale in agriculture. The effects of OP on biochemical processes related to the cholinergic neurochemistry of the developing nervous system, on nucleic acid, lipid, carbohydrate, as well as protein synthesis. The doses of methylparathion which are administered orally to pregnant rats include amounts well below those which cause obvious manifestations of toxicity, both in the mother as well as the fetuses. Preliminary studies involved appropriate dose determinations and the development of the antibody titer system required for future steps in the study.

The study of the pharmacokinetics and toxicities of kepone in workers previously exposed to this compound is continuing. A comprehensive health status reexamination of 12 former kepone workers was completed. All previous manifestations of toxicity have been resolved. Neurologic exams for normal liver size and function was normal and sperm counts were either slightly low or normal. Kepone was undetected in blood or buttock fat. However, four of eight fat biopsies revealed kepone alcohol in the range of 50 to 100 ng/gm. This suggests that kepone alcohol and metabolite of kepone produced by humans, but not by rat liver, is sequestered in human tissues even though the parent compound was removed by natural or by cholestyramine-stimulated processes. The gerbil was identified as a suitable animal model of kepone metabolism in man. Kepone alcohol was identified in the bile, liver, and in the stool of kepone treated gerbils. It also appears as if the gerbil, like man, has a nonbiliary mechanism for excretion of kepone that is inhibited by the presence of bile in the intestine. Finally, the unusually high level of kepone in blood as compared to fat was found to be due to selective binding of kepone to albumin and to high density lipoproteins. This may also explain preferential uptake of kepone by the liver.

EPIDEMIOLOGY

An improved method for estimating attributable risks in various types of epidemiologic studies was developed. Attributable risk is a key index often employed by epidemiologists as an aid in making public health policy decisions.

The attractiveness of this method stems from the fact that it takes into account not only the relative risk of developing a disease due to exposure of some environmental agent, but also the absolute proportion of the population actually exposed to that agent. These efforts have resulted in the development of a method of confidence interval estimation which provides a shorter and more reliable confidence interval than any currently available.

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