NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Annual reports

FY 1983 ANNUAL REPORT

October 1, 1982 through September 30, 1983

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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

In April of this year, the first scientists from the Intramural Research Program occupied laboratory space in the Institute's new building. Since that time there has been a continuous flow of scientific and support staff into the laboratory modules. Occupancy of the new laboratories was completed by the end of August and the leasing of the Northrop Laboratory has been terminated.

Even with the temporary disruption of laboratories preparing to move and moving there has been no disruption to scientific output. Realignments within the Laboratories of Genetics, Chemistry and Biophysics are underway to bring these programs into sharper focus for more effective scientific progress.

Within the Laboratory of Genetics, progress has been made to provide a better understanding of the mechanisms of chemical mutation, to develop better mutation detection methods, and to understand the effects of mutations on human health. Investigators have found that within areas of the DNA where there is a naturally high rate of mutations, there are short unpaired nucleotides which loop out of the DNA's helix. This structure is common to several of the lower species and NIEHS geneticists are looking at mammalian cell DNA to see if the structure is present there as well. At a molecular level, scientists are attempting to define the types and amounts of variability in populations of eukaryotic organisms, while other studies have involved the spontaneous mutations of the white locus in Drosophila, which are the results of insertions of transposable sequences of DNA of unknown origin and function.

During routine screening for mutations in mice by the Laboratory's mutagenesis screening program scientists discovered a mutant gene in mice similar to the one that causes Beta thalassemia (Cooley's Anemia) in humans. The discovery has led to the development of a mouse strain that can be used for in vivo research on this heritable disease. The mutation, discovered in association with scientists outside the Institute, is designated Hbb^{th-1} and represents the only available animal model for studying Beta thalassemia and points out the importance of routine mutagenesis screening.

Within the Institute's Extramural Program, there have been new discoveries related to effects of air pollution from burning coal and the undesirable effects of kerosene space heaters on indoor air. Research at the Environmental Health Sciences Center at MIT has shown that when coal is burned when the humidity is high, two pollutants--sulfur dioxide and zinc oxide--combine to form aerosol by-products that are more toxic than either of the compounds separately. The particles, which are coated with sulphuric acid, sulfates or sulfites, when they are inhaled by laboratory animals, restrict the amount of oxygen absorbed in the blood. This discovery could lead to modifications in power plant design that would further protect human health from the dangers of smoke stack effluent. Studies at the Yale School of Public Health have found that under certain conditions indoor kerosene space heaters can create levels of nitrogen oxide, sulfur dioxide, carbon monoxide and carbon dioxide that exceed ambient air quality standards set for outdoors under the Clean Air Act and in some instances exceed OSHA standards for allowable workplace exposures.

A new research development award, the Clinical Investigator Award in environmental health research, was announced this year. This five-year award is designed to provide intensive guided research experience for clinicians. It will provide up to three years of guided research experience under an established investigator plus two years of additional research support during which time the awardee will be expected to develop a program of clinical research within his or her own department.

Research is being conducted under an Interagency Agreement with the Lawrence Livermore Laboratories to determine how cooking various types of foods produces mutagens which might be precursors to cancer. Researchers found that protein rich foods such as beef, pork, and veal become highly mutagenic when grilled or broiled at high temperatures. Knowledge of the relationship between mutagenesis and carcinogenesis increases, and as we combine this information with what we know about the correlation of rates of different types of cancers and different diets around the world, our increased understanding of food chemistry may be useful in developing diets that will lower our risk from some cancers.

A major portion of the Institute's efforts applied to the National Toxicology Program through its Toxicology Research and Testing Program is extending the endpoints of its bioassays to include information on hepatotoxicity, nephrotoxicity, teratogenicity, mutagenicity, toxic effects on the immune system, lungs, etc. Studies are now routinely performed to help understand the absorption, distribution, and excretion of chemicals at various concentrations. NTP also conducts several types of short-term tests to determine the chemical's effect on the genome.

Traditional testing practices are being examined to determine if there are ways to reducing their costs without compromising quality.

The Breast Milk and Formula Project, an epidemiologic study within the State of North Carolina on the effects of PCBs and DDE on the offspring of over 800 nursing mothers, is producing some interesting information. Some of the mothers participating in the study had been accidentally exposed to PCBs illegally dumped along highways in North Carolina. While the overall level of PCBs found in their milk was not different from what is normally found, one particular PCB in their milk was identified as being similar to that dumped along the roadway, indicating that a small amount of the chemical had likely been absorbed from the spill.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement

During FY 1983 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. The 10th Joint Conference of the Panel was held in Honolulu, Hawaii, February 7-9, 1983 on the topic of Population Monitoring: Methods and Applications. A total of 5 sessions were held on the topics: (1) Evaluation of Reproductive Effects and Spontaneous Abortions, (2) Evaluation of Chromosome Damage, (3) Urine and Semen Anaysis, (4) Population Monitoring: New Methods and Surveillance and (5) Population Monitoring and Future Directions.

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 Thonon, France, April 16-22, 1983. The meeting was devoted to planning of future programs of the Commission and proposed workshops on Retinoblastoma and Down's Syndrome. There were discussions regarding relations between the International Program for Chemical Safety (IPCS) and ICPEMC and future cooperative activities.

Egypt

At the invitation of the Department of Botany, Faculty of Agriculture, University of Zagazig, Zagazig, Egypt, the ADG served as visiting professor from October 29 - November 15, 1982. He presented a series of lectures at the University of Zagazig as well as in Cairo at the National Research Center, Ain Shams University and Cairo University. He also consulted with faculties and researchers regarding possible participation in the IPCS.

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 conference on "Genetic Consequences of Nucleotide Pool Imbalance" which was held at NIEHS on March 9-11, 1983. Participation in the meeting included several NIEHS staff, as well as Foreign and American researchers active in this new area of study. Proceedings of the conference are in the process of being published.

The Proceedings of the NIEHS Workshop on "Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk" were published in July, 1983. The book is edited by the ADG and Dr. William Sheridan.

Seminars

The ADG, in collaboration with Intramural Research Program staff, has developed a seminar series entitled "New Frontiers in Genetics" for the purpose of inviting scientists whose research is the vanguard of advances in their field of genetics to present their work to the NIEHS scientific staff. Lectures dealing with molecular genetics, advanced cytogenetics, and monoclonal antibodies have been given by such eminent scientists as William Engels, Jorge Junis, and Paul Lohman.

Committees

Subcommittee on Environmental Mutagenesis - The ADG continues to chair the DHHS/CCERP Subcommittee on Environmental Mutagenesis. Topics covered included: a review of the NIEHS portion of the National Toxicology Program, Genetic Effects of Ethylene Oxide, Genetic and General Toxicology of Benzene, The Love Canal Cytogenetics Study, and Mutagens from the Cooking of Food. These and other current issues of importance to government agencies concerned with genetic toxicology will continue to be addressed by the Subcommittee.

The Committee for Hazard Evaluation and Risk Estimation - which was organized and is chaired by the ADG, continues to meet on a monthly basis to review experimental methodologies and techniques, current literature and recent results, for their suitability for estimation of risk.

Collaborative Studies

WHO - International Program for Chemical Safety

The ADG is chairman of a working group of the International Program for Chemical Safety (IPCS) sponsored by the World Health Organization, the United Nations Environmental Program and the International Labor Organization. A meeting of the working group was held in Geneva, Switzerland, May 16-18, 1983, to conduct a review of the status of Part I (in vitro)

and to further plans for Part II (in vivo) of the IPCS study on short-term tests for genotoxicity and carcinogenicity. Plans for a meeting of the investigators participating in Part I, to be held at St. Simons Island, Georgia in October, 1983, were developed. This meeting will consist of a series of assay systems workshops to evaluate the results which have been obtained and to prepare workgroup reports.

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. New York Academy of Sciences Conference on Cellular Systems for Toxicity Testing, New York, NY, October 5-7, 1982, "The Role of Neurospora in Evaluating Environmental Chemicals for Mutagenic Activity".

The following five lectures were presented by the ADG during his Egyptian visit, October 29 - November 15, 1982:

2. "The Role of Neurospora in Evaluating Chemicals for Mutagenic Activity".

3. "The Use of Tradescantia in Monitoring for Mutagenic Air Pollutants".

4. "Interaction and Mutation Induction in Wild-type and Repair Deficient Haploid Strains of Neurospora crassa".

5. "International Program for the Evaluation of Short-term Tests for Carcinogenicity (IPESTTC)".

6. "Mutagen Specificity in Wild-Type and Repair Deficient Two-Component Heterokaryons of Neurospora crassa".

7. Mary Ann Swetland Program in Medicine and Human Behavior, Case Western Reserve University, Cleveland, OH, January 10, 1983 "Evaluation of the Utility of Short-term Tests for Carcinogenicity and Mutagenicity".

8. 14th Annual Meeting of Environmental Mutagen Society, San Antonio, TX, March 3-6, 1983, "Induction of ad-3 Mutants in Nucleotide Excision-Repair Deficient Strains Provides Evidence for Qualitative Differences in the Spectrum of Genetic Alterations from that found in Wild-Type Strains of Neurospora crassa".

9. NIEHS Conference on Genetic Consequences of Nucleotide Pool Imbalance, NIEHS, Research Triangle Park, NC, May 9-11, 1983 "Base Analog Mutagenesis in Neurospora".

10. The Gordon Conference on Genetic Toxicology Bioassays, New London, NH, June 27-July 1, 1983 "The Evaluation of Short-Term Tests for Carcinogens in the International Program for Chemical Safety".

William Sheridan

1. 14th Annual Meeting of Environmental Mutagen Society, San Antonio, TX, March 3-6, 1983, "Effects of Triethylenemelamine (TEM) Exposure on Fetal Germ Cells of Male Mice".

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

The main function of this office remains the evaluation of human health hazards, particularly from chemicals in man's environment, both newly introduced and those already used in agriculture, industry and in the home as components of food, beverages and consumer products, as well as air, water, food and soil contaminants. In addition, the office has given increased attention to the assessment of methods for testing and for the control of exposure to chemicals.

Human exposure can occur directly through skin contact or ingestion or indirectly through environmental media. The chemical form, biological availability and concentration of chemicals may change in their passage through the environment. They may be degraded to less toxic substances or may be transformed to more toxic compounds. They may accumulate selectively in some organisms and their concentrations may become many times higher than those found in contaminated water or soil. On the other hand, they may be eliminated from water or air by various physical processes. Secondary pollutants can be formed by chemical or biochemical reactions. Some chemicals are stable and may persist in the environment for a long time. Examples are polychlorinated dibenzodioxins which occur as contaminants of some herbicides such as 2,4,5-trichlorophenoxy acetic acid, and polychlorinated biphenyls.

Mutagenic potential is well-established for many chemicals, mainly as a result of in vitro testing. Further research, particularly on the mechanisms of DNA repair inhibition, is needed to assess the possible role of environmental chemicals in human genetic disease. Pathophysiological processes induced by exposure to chemicals may involve a complex chain of changes that develop gradually and are not detected for a long time. A long latency period characterizes the effects of some substances so that the time from the first exposure to the development of a clinically detectable cancer can exceed 20 or 30 years. In addition, man is rarely exposed only to a single chemical; a better knowledge of the effects of mixtures and of host and environmental factors that may modify the outcome of exposure would greatly improve the possibility of controlling diseases that are either induced or promoted by chemicals.

In most cases, the assessment of health hazards is based on data from animal experiments. This presents difficulties because methods for testing are not uniform, and animals may not respond to chemical exposure in the same way as human subjects; groups of animals exposed in laboratory tests are small and homogeneous compared to large heterogeneous human populations which include very young and very old persons, those that are healthy and those that suffer from various diseases. Thus, the assessment of existing methods for testing chemicals and identification of their short-comings is essential for developing better methods for risk estimation in humans. This applies in particular to methods for testing and evaluating mixtures of chemicals.

To assist in the selection of chemicals for the National Toxicology Program (NTP), all members of OHHA and Drs. D.B. Walters, TRTP, and B.A. Fowler, LP, have continued to participate in the NIEHS Subcommittee of the NTP Chemical Nomination and Selection Committee, which has been chaired this year by Dr. Piver. Dr. Vouk has remained a member of the NTP Toxicology Design Committee.

OHHA staff also contributes to the preparation of the Annual Reports on Carcinogens. Because of a prolonged absence of the Assistant Director for Toxicology Coordination, Dr. Vouk coordinated the final review and revisions of the Third Annual Report.

Collaboration with the World Health Organization (WHO) has continued both within the framework of the International Programme on Chemical Safety (IPCS) and other WHO programs in environmental health, NIEHS being a WHO Collaborating Center on Environmental Health Effects. OHHA has been responsible for NIEHS's contributions to and review of IPCS draft evaluations of priority chemicals and toxicological methods. As a WHO temporary adviser, Dr. Vouk stayed three weeks in Geneva in April and prepared a draft working paper on organizational arrangements of the IPCS, and participated in consultation on the same subject held in Geneva in July. Drs. Vouk and Piver participated in a Workshop on methods of toxicological testing and evaluation of mixtures of chemicals in Guildford, England, in August. Dr. Vouk also participated in the Conference on Environmental Research and Management Priorities in the 1980's organized by the Royal Swedish Academy of Sciences in Rättwick, Sweden, in November 1982.

The Associate Director spent most of the fiscal year away from the office due to a series of illnesses. Because of that, he attempted to complete his obligations to other organizations. He completed his term as member of the Scientific Advisory Panel for the Chemicals Industry Institute of Toxicology (CIIT) and terminated his functions as Chairman of the Visiting Committee to the Brookhaven National Laboratory's Medical Division.

His membership on the International Joint Commission's Committee on the Assessment of Health Effects of the Great Lakes Water Quality had to terminate this summer. This year saw the results of a research project carried out by Cornell Univeristy's Drs. Ronald Brickman, Sheila Jasanoff and Thomas Ilgen. A book entitled Chemical Regulation and Cancer: A Cross-national Study of Policy and Politics was published for which the Associate Director had served as advisor.

The work of OHHA was supervised by Dr. Warren T. Piver as Acting Associate Director for the first half of the fiscal year and by Dr. Velimir B. Vouk as Acting Director, OHHA, for the second half.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20002-11 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Technology Forecasting and Technology Assessment

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Warren T. Piver Chemical Engineer OHHA NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Because subsurface burial of industrial chemicals has been a common method of waste disposal, a mathematical model describing the simultaneous transfer of heat, moisture, and chemicals in unsaturated and saturated soils was developed. By subdividing the soil into layers, heat, mass, and moisture balances can be constructed for each layer transforming a continuous non-linear model into a model that is more easily solved. This model has been used to examine the transport of PCBs in soils with different adsorptive capacitites.

The design of incinerators for PCBs has been analyzed to determine the conditions necessary for destruction without formation of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs). Formation of PCDFs and PCDDs is eliminated by maintaining in the combustion chamber sufficient temperatures, residence times, turbulence, and oxidizing conditions that are similar to the design features of an isothermal plug flow reactor.

The study of the mobilization of arsenic by industrial processes focussed on coal combustion for electric power production and new uses of coal for liquefaction and gasification. Arsenic is vaporized during combustion and deposited largely on submicron particles. Much of this material passes through most air pollution control equipment. Analyzing data for partitioning of arsenic in liquefaction and gasification indicated that in liquefaction the potential for formation of organometallic compounds of arsenic is high. For gasification the atmospheric release of arsenic deposited on submicron particles is the most probable route of environmental entry.

This project will be reoriented during the next Fiscal Year and possibly divided into several separate projects.

Principal Investigator and All Other Personnel Engaged on the Project:

Warren T. Piver	Chemical Engineer	OHHA	NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA	NIEHS
Herbert S. Posner	Pharmacologist	OHHA	NIEHS
Velimir B. Vouk	Visiting Scientist	OHHA	NIEHS
Naomi Jean Bernheim	Microbiologist	OHHA	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: During the past year major attention has been given to waste disposal technologies, and to methods for selection of chemicals for mutagenicity testing based on molecular structure and biological relationship activity.

In the analysis of chemical transport in soils, migration of chemicals in the unsaturated zone of the soil has been studied during the past year. The soil column is subdivided into layers. Heat mass and moisture balances are constructed about each layer, transforming a continuous model into a stagewise model -- a situation that is mathematically less difficulty to solve. The model makes it possible to simulate many different field conditions and allows greater flexibility in specifying soil and chemical properties. It has been used to analyze transport and transformation of chloroaliphatic and chloroaromatic chemicals under a variety of external and internal conditions.

In conjunction with the World Health Organization and the International Programme on Chemical Safety, a project on groundwater quality has been initiated. To determine the present state of knowledge and provide the basis for recommendations for program development, three workshops are being planned for the next year on transport, on groundwater sampling and analysis, and on toxicity due to multiple chemicals found in groundwaters.

The design of incinerators for disposal of PCBs has been analyzed to determine the conditions necessary for destruction without formation of polychlorinated dibenzofurans (PCDFs) and dibenzodioxins (PCDDs). Methods have been proposed to determine the proper size and operating temperatures that produce high levels of destruction. Formation of PCDFs and PCDDs is eliminated by maintaining in the combustion chamber sufficient temperatures, residence times, turbulence, and oxidizing conditions. These conditions are achieved with incinerator designs that approximate the design features of isothermal plug flow reactors.

Combustion of coal for space heating and electical power production was examined for factors that effect emissions of trace elements in coal. Elements that occur as sulfides in coal -- V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Sr, Mo, Cd, Sb, Ba, and Pb--are preferentially deposited on submicron fly ash particles and pass through many air pollution control devices.

The mobilization of arsenic by natural and industrial activities was analyzed. Attention focused on coal combustion for power production and utilization of coal for gasification and liquefaction in the future. In addition, copper smelting and geothermal power were included in this analysis. Because of the volatility of arsenic at the temperatures and pressures used in these techniques, the potential for formation of many new chemical forms of arsenic as well as the well-known inorganic forms of the element is high.

Assistance in selecting chemicals for the NTP mutagenicity testing program has resulted in recommendations on sulfa drugs, nitropolynuclear aromatics, selected groups of industrial chemicals, and a proposal for the development of a test system based on the reduced fidelity of DNA synthesis in the presence of metal ions. During the past year, test results for nitro aromatics and aromatic amines have been analyzed to determine arrangements of groups that produce the most activity in the Salmonella test system. The most active arrangements include nitro groups along with other electrons withdrawing groups in the ortho and para position. Using this information, lists of commercially important compounds are being examined for chemicals with similar characteristics that have not been tested.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These activities will continue to provide necessary information about the chemical characteristics and the entry of commercially important chemicals into the environment, and to identify gaps in knowledge with regard to toxicity, bioaccumulation, and environmental transport and transformation. Such information provides background information for toxicity evaluations within the Institute and for the projects of NTP.

PUBLICATIONS

Piver, W.T.: Mobilization of arsenic by natural and industrial processes. In Fowler, B.A. (Ed.): The Biological and Environmental Effects of Arsenic. Amsterdam, Elsevier/North Holland Press, 1983 (in press).

Piver, W.T., and Lindstrom, F.T.: Simulating transports of organic chemicals in saturated/unsaturated soils. In Proceedings of the International Conference on Ground Water Contamination with Organo-Chlorine Compounds of Industrial Origin, 26-29 January 1983, Milano, Italia (in press).

Piver, W.T.: Waste disposal technologies for PCBs. Environ. Health Perspect. (in press).

Piver, W.T., and Lindstrom, F.T.: Subsurface burial of organic chemicals and groundwater contamination. J. Hazard. Wastes (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20003-10 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Preventive Surveillance of Environmental Chemicals for Toxic Potential

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Herbert S. Posner Pharmacologist OHHA NIEHS

COOPERATING UNITS *(if any)*
Cellular Genetic and Toxicology Branch, TRTP/NIEHS

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Some host factors and environmental conditions may modify the effects of methanol, paraquat and aspartame. Methanol is a hazard to children when it is applied as a body rub for fever or to relieve colic; when accidentally used in formula instead of water; or when abused by older children and adolescents for inebriation or sedation. Inhalation of solvent vapors containing methanol and methylene chloride caused fatal poisoning in an adult. Methanol is absorbed through skin about three times as fast when mixed with gasoline or diesel fuel. Paraquat is more toxic to experimental animals when co-administed with many other chemicals. There are reports of human fatalities from paraquat in field work after heavy exposure of the skin (or broken skin); spraying in confined locations; or by unclogging sprayguns by sucking or blowing. Helicopter spraying of illicit Cannabis may be hazardous if persons on the ground are not warned; if they brush against wet foliage; or if interspersed or nearby food crops are eaten. There are reports of paraquat effects on DNA and mutagenicity. Aspartame may be hazardous to phenylketonuric individuals, very young infants, and patients with severe liver impairment. A diketopiperazine-type conversion product is more toxic in animals than is aspartame. Formation of the product is faster at increased temperature, moisture, acidity or alkalinity, and some aspartame preparations have designated shelf lives of about half-a-year. Other Activities: Clinical and epidemiological literature cannot be evaluated if the sex of subjects is not clearly defined. A case for clarification in medical, scientific and technical literature has been made. Data from the National Toxicology Program are being evaluated for the relationship between structure and toxicity.

Some activities of this project will be terminated or reoriented during the next Fiscal Year.

Principal Investigator and All Other Personnel Engaged on the Project:

Herbert S. Posner	Pharmacologist	OHHA	NIEHS
William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
Warren T. Piver	Chemical Engineer	OHHA	NIEHS
Naomi Jean Bernheim	Microbiologist	OHHA	NIEHS
Errol Zeiger	Microbiologist	CGTB	NTP

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

The objective of the project is to identify conditions and host factors that increase health hazards of specific chemicals, including simultaneous exposures to other chemical and physical agents, and behavioral or genetic factors.

Methanol is usually considerd a hazard to adults when ingested, but it may be also a hazard to young and older children, and to adolescents. Exposure can be fatal when methanol is used as a body rub in young children, or when it has been mistaken for water in preparing liquid formula. Older children and adolescents may abuse methanol by ingestion as a substitute for ethanol. There do not appear to be many cases of abuse by sniffing, as with some aromatic and chlorinated hydrocarbons. A review of methanol intoxications in children and adolescents is in preparation. Ethanol is known to alter metabolism and effects of many chemicals, and methanol may have a similar action. Very slow metabolism of methanol may increase this hazard. Inhalation of vapors of a methanol and methylene chloride mixture, used as solvent, caused fatal poisoning in a sensitive individual. This was due to accumulation of carbon monoxide from metabolism of methylene choride and secondary formation of methemoglobin. Rate of absorption of methanol through skin may increase up to three times, if methanol is contained in a mixture with gasoline or diesel fuel. Literature search is being made for other examples of such interactions.

Paraquat. Animal experiments have indicated that simultaneous exposures to many other chemicals increase the toxicity of paraquat. For this reason, simultaneous exposures should be avoided in persons exposed to paraquat until more information is obtained. Several reports are available on paraquat poisoning resulting from heavy exposure through both intact and broken skin, from exposures due to spraying in confined spaces, and from sucking or blowing on sprayguns to unclog them. Helicopter spraying of illicit Cannabis fields with paraquat can be hazardous to individuals on the ground if they are not warned, or to persons brushing against the wetted foliage, or if interspersed or neighboring food crops are eaten. There are several reports that paraquat is mutagenic and has effects on DNA in experimental animals.

Aspartame. This low calorie sweetener could be a health hazard to persons suffering from phenylketonuria, to infants, and to persons with severe liver disease. High temperature and humidity, acids and bases, and long storage may significantly increase the amount of a diketopiperazine derivative, a decomposition product of aspartame that is more toxic.

Chemical structure and bilogical activity. A study of National Toxicology Program Salmonella test results for nitro-compounds has been initiated to evaluate chemical structure-biological activity relationships. This may enable the identification of further nitro-compounds that require testing. This study will be expanded to cover some other classes of organic compounds.

Other activities. A review of some published papers on clinical and epidemiological studies showed that the sex of persons investigated is not always clearly specified. This is a deficiency often found in reports on human studies.

Response has been provided to a request for information on long-standing health problems in a family, apparently associated with malfunction of a solar heating system and to several other requests.

A partial analysis of the "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" has been made.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Modification of effects of chemicals by host and environmental factors is a major problem in application of animal data to humans who are rarely exposed to single substances and who are inhomogeneous as regards various host factors.

PUBLICATIONS

Posner, H.S.: Unambiguous reporting of the sex of human beings. Lancet I: 175-176, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20007-06 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification and Health Hazard Evaluation of Industrial and Food Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
William Jurgelski, Jr. Medical Officer OHHA NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Reproductive and Developmental Toxicology (LRDT/NIEHS)

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

As a member Dr. Jurgelski participated in meetings of the NIEHS Subcommittee of the NTP Chemical Nomination and Selection Committee. As part of this assignment, a list of the chemicals used in the microelectronics industry was compiled and presented to the subcommittee.

A review of chemical toxicity in utero was discontinued after a paper that adequately summarized information in the area was published by other investigators.

Two reviews on methanol toxicity have been initiated in collaboration with Dr. H. Posner.

This project may be reoriented during the next Fiscal Year.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA	NIEHS
Herbert S. Posner	Pharmacologist	OHHA	NIEHS
Jean N. Bernheim	Microbiologist	OHHA	NIEHS
Warren Piver	Chemical Engineer	OHHA	NIEHS
David Pasquini	Air and Industrial Hygiene Engineer		RTI
Larry Laird	Engineer		RTI

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

1. Dr. Jurgelski continued to be a member of the NIEHS Subcommittee of the NTP Chemical Nomination and Selection Committee. Within this framework he compiled a list of the chemicals in use in the microelectronics industry. The chemicals were identified through consultation with staff of the Research Triangle Institute and from the reports of several Governmental and industrial organizations including the Division of Occupational Safety and Health of the State of California Department of Industrial Relations, the Semiconductor Industry Association, and the National Institute of Occupational Safety and Health. This list will be expanded in collaboration with OHHA and RTI staff into a review of the chemical health hazards in the industry either for publication or for internal Institute use.

2. Work begun on a review in collaboration with Drs. Robert Dixon and Robert Pratt, tentatively titled: "Chemical Toxicity in Utero, A Review of Targets, Mechanisms and Methods" was discontinued because review of the same topic was published by other investigators.

3. Two reviews have been initiated in collaboration with Dr. Posner, OHHA. These papers represent the completion of his on-going work on methanol toxicity. The available information on two aspects of methanol toxicology is being summarized in the reports on "Methanol as a pediatric toxin" and "The comparative toxicity of methanol, ethanol, isopropyl alcohol and gasoline."

4. A review of published information comparing the use of different animal models in toxicology has been initiated to determine whether a report oriented toward specific applications of laboratory animals is warranted.

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 and 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20008-06 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Marsupial Model in the Identification and Evaluation of Environmental Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
William Jurgelski, Jr. Medical Officer OHHA NIEHS

COOPERATING UNITS *(if any)*
Office of Health Hazard Assessment

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 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 *(Use standard unreduced type. Do not exceed the space provided.)*

An invited review describing the use of the marsupial as a model in perinatal carcinogenesis research has been published.

There were no requests this year for consultation from the laboratories to which the Principal Investigator had provided information and advice on use of the marsupial model in on-going or proposed studies.

This project will be terminated upon completion of a paper in preparation.

Principal Investigator and All Other Personnel Engaged on the Project:

William Jurgelski, Jr.	Medical Officer	OHHA NIEHS
Pearlie M. Hudson	Physical Sci. Tech.	LET NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA NIEHS
L. E. Zimmerman	Ophthalmic Pathologist	Armed Forces Institute of Pathology (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

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

1. Invited Papers

   a. An invited review entitled "An Alternative Animal Model for Perinatal Carcinogenesis" has been published in Biological Research in Pregnancy and Perinatology. This paper describes the differences in developmental biology between marsupials and eutherian animals that provide alter- results of studies conducted by the Principal Investigator in which dysontogenic tumors with close morphologic and biologic resemblance to human lesions were produced in the neonatal opossum. The possibilities that the marsupial offers for transplacental carcinogenesis studies as well as the potential of pre- and post-natal life support systems in enhancing the use of the marsupial model are considered. Finally, the choice of marsupials is described.

   b. An invited review entitled "The Marsupial as an Animal Model in Space Biology" is in preparation for Biological Reviews.

2. Collaborative Studies and Consultations.

   a. Dr. Jurgelski aided in establishing a small colony of opossums at the Max Planck Institute of Biochemistry in Munich, West Germany, for the purpose of reproducing gangliogliomas of the brain for in vitro culture. (See annual reports for FY 74-81.) This project is being terminated because the collaborator, Dr. Bernard Hamprecht, is leaving his post at the Institute.

b. There have been no requests for consultation from several laboratories in the United States and in Belgium to which Dr. Jurgelski provided information and advice on use of marsupial models in connection with on-going or proposed studies.

This project is being terminated except for completion of the invited review in preparation. Dr. Jurgelski will continue to consult on an informal basis with investigators who request information and/or advice on the use of marsupials in biomedical research.

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 materna! 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.

PUBLICATIONS

Jurgelski, W.: An alternative animal model for perinatal carcinogenesis. Biological Research in Pregnancy and Perinatology 4(1): 3-16, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20009-05 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification of Potential Environmental Health Hazards

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Naomi Jean Bernheim Microbiologist OHHA NIEHS

COOPERATING UNITS *(if any)*

Cellular Genetics and Toxicology Branch, Toxicology Research and Testing Program (TRTP)

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

A new project was initiated on the analysis of mutagenicity of 155 chemicals that were tested by the EMTDP Laboratory for mutagenicity using the Salmonella test and grouped according to structural similarities.

Other activities included compiling toxicological data for insertion in data base records and profiles of chemicals identified in waste dumps throughout the United States; collecting and documenting carcinogenicity information for the NTP Annual Reports on Carcinogens; and compiling information on chemical/chemical interactions, chromosome breakage and/or sister-chromatid exchange in human cells, and the mechanism of action of oncogenes.

Principal Investigator and All Other Personnel Engaged on the Project:

Naomi Jean Bernheim	Microbiologist	OHHA NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA NIEHS
Errol Zeiger	Microbiologist	CGTB/TRTP/NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

Chemical Structure--Mutagenic Activity Relationship: An analysis was initiated of EMTDP Salmonella mutagenicity test data on 155 chemicals grouped according to structural similarities. An "estimated maximum slope" was calculated for the highest number of revertants/plate on the dose curve before toxicity became evident. The data were tabulated to show the differences between an activated (rat and hamster liver) and non-activated compound and the differences between the four Salmonella strains. This information on relative mutagenicity of similar compounds can be used in selection of chemicals for further testing by NTP.

Carcinogenicity Data for NTP's Annual Reports on Carcinogens: Carcinogenicity data were collected, documented, and summarized for inclusion in the Third Annual Report on Carcinogens. There were 88 compounds in the Second Annual Report on Carcinogens published December 1981, which were updated; 28 compounds were added to Third Annual Report to be published in 1983.

Human Chromosome Breakage and/or Sister Chromatid Exchange: Different types of chromosome abnormalities associated with specific chemical exposures have been searched for, as well as their relationship to mutagenicity and/or carcinogenicity. Refined techniques for chromosome isolation and molecular characterization, such as the use of interspecies hybrid cells, flow cytometry and DNA cloning in bacteria, provide large numbers of chromosomes for detailed biochemical analysis. Chromosomal deletions, translocations and gene amplifications are known to be positively associated with specific malignancies.

Activation of Oncogenes: An in-depth review has been initiated on the mechanism of activation of oncogenes, in particular, (1) the activation of the oncogene by transposons; (2) transposition of the gene to another chromosome; (3) mutations in the DNA of that gene, and (4) activation of specific oncogenes in specific chromosomes.

Chemical/Chemical Interactions: This is a long-term project which has been initiated because most human exposures to chemicals involve mixtures. An open-ended file has been developed for several years to collect specific types of interactions which are filed to be retrieved by either the compound itself or the test system used. Files are also maintained relating to co- and/or anti-mutagenicity, carcinogenicity and teratogenicity of compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Information available in the literature can often indicate potential hazards of new chemicals which are bearing close relationships to other better known chemicals. This may help identify chemicals which require testing. Another aspect of this project, interactions and exposure to mixtures, is important for many research projects of the Institute.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 20010-03 OHHA

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification and Mechanism of Action of DNA Repair Inhibitors

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Naomi Jean Bernheim Microbiologist OHHA NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director (OD)

SECTION

Office of Health Hazard Assessment (OHHA)

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to identify and evaluate potential DNA repair inhibitors by an in-depth search of the literature; to find out if specific mechanisms exist for DNA repair inhibition; and to evaluate whether this information can be practically applied. The study includes a comprehensive review of interference with DNA repair by chemical, physical, and genetic factors. Cell death, delayed lethality, loss of proliferative capacity, and mutagenesis are expressions of unrepaired DNA changes, misrepaired nucleotide sequences, delayed repair, or copying errors during the semi-conservative DNA replication in the cell cycles following the application of a mutagenic agent.

It has been shown that DNA repair may be interfered with through different biochemical mechanisms depending on the type of DNA damage and the interfering agent. One new development is the role of poly (ADP-ribose) polymerase in the repair process as a suppressor of DNA synthesis, providing critical time for repair to take place prior to DNA replication. Interference with DNA repair is one of several mechanisms proposed to explain co-mutagenicity.

Principal Investigator and All Other Personnel Engaged on the Project:

Naomi Jean Bernheim	Microbiologist	OHHA NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: DNA is vulnerable to physical and chemical attack but effective enzymatic mechanisms for repairing such damage exist in procaryotes and eucaryotes. Cells that are genetically defective in a particular repair pathway are more easily killed, or subject to mutations, or transformation by radiation or chemicals. However, even in a genetically non-defective cell, DNA repair may not be as efficient as required. Lack of DNA repair capacity has also been found to be due to chemical insults.

Many agents referred to as DNA repair inhibitors are as active or more active in suppressing replicative synthesis and RNA and protein synthesis. Mechanisms involved in DNA repair inhibition include inhibition of protein synthesis or alterations in energy metabolism, direct interaction with DNA by intercalation or binding, effects on precursor pathways and/or enzymatic cofactors and modulation by physical factors. Interference with DNA repair may be one of several mechanisms underlying "co-mutagenicity."

Metal ions are required for every aspect of genetic information transfer and certain metal salts may act as co-mutagens by affecting the repair of damage to DNA caused by another agent. Trace metals may replace the essential element in an enzyme and result in a less effective enzyme action (e.g. decreased fidelity of DNA polymerase). A newly discovered factor is the role of poly (ADP-ribose) polymerase in the repair process. The functinal role of this polymer in DNA repair may be to suppress DNA synthesis and provide critical time needed for repair to take place prior to DNA replication. Inhibitors of poly (ADP-ribose)polymerase potentiate the cytotoxicity of alkylating agents and ultraviolet radiation in rodent cell lines.

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 (New York City, November 2, 1981). The symposium proceedings have been published.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information available in the literature can often provide indication of potential hazards from exposure to new chemicals which have a close structural or functional relationship to other better known chemicals. It has been already demonstrated that the application of DNA repair inhibitors increases the beneficial effects of chemotherapy. However, DNA inhibitors may also prove detrimental if they interact synergistically with mutagens. DNA repair assays are gaining credibility as a component in a battery of short-term predictive tests for mutagens/carcinogens.

PUBLICATIONS

Bernheim, N.J., and Falk, H.L.: Chemical, physical and genetic factors interfering with DNA repair -- a review. J. Amer. Coll. Toxicol. 2(3): 23-54, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 20011-03 OHHA

PERIOD COVERED
October 1, 1982, to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comprehensive Evaluations of Biological Effects of Chemicals on Health

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Velimir B. Vouk Visiting Scientist OHHA NIEHS

COOPERATING UNITS *(if any)*
Biometry and Risk Assessment Program (BRAP/NIEHS), Laboratory of Reproductive and Developmental Toxicology (LRDT/NIEHS), WHO/IPCS Inter-Regional Research Unit (IRRU) at NIEHS and some 20 research institutions in the USA and other countries.

LAB/BRANCH
Office of the Director (OD)

SECTION
Office of Health Hazard Assessment (OHHA)

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

During the year under review, the emphasis has been on the toxicological assessment of selected metals and metal compounds. Other activities included methods for assessing the effects of chemicals on reproductive function of mammals, other vertebrates, invertebrates, plants, and microorganisms; methods for estimating exposure, and quantifying risk in humans and damage to non-human biota and ecosystems; methods for toxicological testing and evaluation of mixtures of chemicals; and methods for evaluating impairment of physiological functions caused by exposure to chemicals.

The objective of this project, implemented partly in cooperation with the WHO/UNEP/ILO International Programme on Chemical Safety (IPCS), is to evaluate biological effects and health hazards of selected chemicals and methods for toxicological studies. This project may be reoriented during the next Fiscal Year and possibly divided into two projects.

Principal Investigator and All Other Personnel Engaged on the Project:

Velimir B. Vouk	Visiting Scientist	OHHA NIEHS
Hans L. Falk	Associate Director for Health Hazard Assessment	OHHA NIEHS
Warren Piver	Chemical Engineer	OHHA NIEHS
William Jurgelski, Jr.	Medical Officer	OHHA NIEHS
Herbert S. Posner	Pharmacologist	OHHA NIEHS
Naomi J. Bernheim	Microbiologist	OHHA NIEHS
Janet Guthrie	Microbiologist	OHHA NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

Toxicology of metals: Revision and updating of ten chapters of the Handbook on the Toxicology of Metals (Elsevier/Biomedical Press, Amsterdam, New York, London) edited by Lars Friberg, Gunnar Nordberg and Velimir B. Vouk, published in 1979 and reprinted with corrections in 1980, was completed. The second edition, to be published under the title "Toxicology of Metals" is a comprehensive review of biological effects of some 30 metals. Dr. Vouk is author or co-author of the introduction and of chapters on general chemistry of metals, sampling and analytical methods, mathematical and statistical aspects of dose-effect and dose-response relationship, standards and criteria, bismuth, germanium, selenium, tellurium, and vanadium. Other NIEHS participants in the project are Dr. Goyer, Deputy Director NIEHS, and Dr. Fowler, LP/ NIEHS.

A paper by Drs. Vouk and Piver on metallic elements in fossil fuel combustion products, amounts and form of emissions, and evaluation of carcinogenicity and mutagenicity of metals was published in Environmental Health Perspectives in January 1983, and so was the Consensus Report of a Symposium held in Stockholm in February 1982, in which Dr. Vouk was a co-author of the section on epidemiology.

At the invitation of the Royal Swedish Academy of Sciences, Dr. Vouk prepared a paper on the release into the atmosphere of metals and metal compounds and attended the Conference on Environmental Research and Management Priorities for the 1980's convened in Rättwick, Sweden, November 23-26, 1982. The results of the Conference are summarized in Ambio, Volume XII, Number 2, 1983.

Methods for Estimating Exposure and for Quantifying Risk in Humans and Chemical Injury to Ecosystems: The joint report and 28 contributed papers of the second study of the WHO/UNEP/ICSU-SCOPE Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC). The first draft of the monograph was prepared at the workshop in Rome in July 1982. The scientific editing was completed by Dr. Vouk in collaboration with Dr. Hoel, Director BRAP/NIEHS and Drs. Butler and Peakall (Canada). Drs. Barrett and Thomassen, LPFT/NIEHS, and Dr. Wilcox, BRAP/NIEHS, also participated in the study.

Methods for Testing for and Evaluating the Toxicity of Mixtures of Chemicals: This third study of SGOMSEC was initiated by the end of 1982 and continued at a workshop organized at Guildford, England, in August 1983. The participants from NIEHS were Drs. Vouk and Piver, OHHA, and Dr. David G. Hoel, Director, BRAP.

Methods for Assessing the Effects of Chemicals on Reproductive Functions: The Joint Report and 24 papers prepared for a SGOMSEC workshop held in Ispra, Italy, in May 1981, were edited by Drs. Vouk and Sheehan, and published as SCOPE 20/SGOMSEC 1 monograph. Dr. Robert Dixon, Chief LRDT/NIEHS, assisted in editing the section on mammalian male reproduction.

Principles and Methods of Evaluating the Toxicity of Chemicals: The preparation of the second volume of a monograph on toxicological methods continued in collaboration with several scientists from the United States, Bulgaria, Canada, Czechoslovakia, Italy, Netherlands and the Soviet Union. This monograph deals with functional studies of organs and systems, effects on reproduction including embryotoxicity and fetotoxicity, neurological and behavioral studies, the skin and the eye, cumulation and adaptation and modifying factors. The preparation of the monograph is sponsored by the International Programme on Chemical Safety (IPCS), a joint undertaking of the World Health Organization (WHO), the United Nations Environment Programme (UNEP) and the International Labour Organization (ILO).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Evaluation by international groups of scientists of biological effects of chemicals and of toxicological methods will help in reviewing, and if necessary, reorienting some research activities of the Institute towards objectives that are of wide scientific interest within the United States and internationally.

PUBLICATIONS

Friberg, L., Albert, R.E. Hogan, M., Nelson, N., Speizer, F., and Vouk, V.: Epidemiology. In Holmberg, B., and Alborg, V. (Eds.): The mutagenicity and carcinogenicity of combustion emissions. Environ. Hlth. Perspec. 47: 4-5, 1983.

Parizek, J., Somers, E., and Vouk, V.B.: Assessment of Health Impact of Environmental Chemicals. In Gilad, A., and Tarkowski, S. (Eds.): Risk Assessment. Copenhagen, World Health Organization, Regional Office for Europe, 1982, pp.161-176.

Vouk, V.B.: Release into the Atmosphere of Metals and Metal Compounds. Proceedings of the Royal Swedish Academy of Sciences Conference on Environmental Research and Management Priorities for the 1980's. Rättvick, Sweden, 23-26 November 1982 (in press).

Vouk, V.B., and Piver, W.T.: Metallic elements in fossil fuel combustion products. Amounts and form of emissions and evaluation of carcinogenicity and mutagenicity. Environ. Hlth. Perspec. 47: 201-225, 1983.

Vouk, V.B., and Sheehan, P.J. (Eds.): Methods for Assessing the Effects of Chemicals on Reproductive Functions, SCOPE 20/SGOMSEC 1, Chichester, New York, Brisbane, Toronto, John Wiley & Sons, 1983, XXV + 541 pp.

Vouk, V.B., Ozolins, G., Hasegawa, Y., and Parizek, J.: Some international activities in environmental monitoring and surveillance. Environ. Monit. Assess. 1: 387-404, 1982.

BATTELLE PACIFIC NORTHWEST LABORATORIES - Richland, Washington
(MIH-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: $122,550

PROJECT DESCRIPTION

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

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

METHODS EMPLOYED: Soils with known properties were amended with labeled and unlabeled isotopes of Ni, Cd, Cr, and Tl. Soybean plants were grown under controlled conditions and mature plants were separated into roots, stems, leaves and beans, and metal contents determined. Hydroponically grown plants were used to study the kinetics of single and competitive ion uptake across root membranes.

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

Neutral organometallic complexes that had been made by the soil microbes were separated from changed complexes by passing the extract through a soil column. Charged complexes were adsorbed to humic and fulvic acid fragments of the soil, and neutral complexes passed through. The chemical characteristics of these neutral organometallic complexes were 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.

From chromotography studies neutral metal complexes appeared to be composed of asparagin and glutamine in a ratio of 3:1. Mixed metal ligand complexes were also detected that had the ability to transport metal in soils and plants. The most frequently observed complexing ligands were glutamine, aspartic acid, and asparagine.

Induction studies designed to determine if plants could metabolically adapt to long-term exposure to Cd^{+2} by changing transport rates and storage patterns were completed. Results indicated that root absorption rates remained constant, whereas long-term induction resulted in saturation of root storage compartments causing greater roots to shoot transfer. Because this is the final year of this program, a summary journal article is being prepared that presents the details of methodology used, the program results and interpretations of data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Metals may be structural elements, stabilizers of biological structures, components of control mechanisms and enzyme activators. Some metals are essential elements and their deficiency results in impairment of biological functions. When present in excess, even essential metals may become toxic. A number of avenues exist by which metals may enter the human food chain. One of the most direct sources is the use of metal containing agricultural chemicals. An equally important source of metal compounds in food are from metal contaminants in fertilizers and fly ash particles from combustion of fossil fuels that are deposited on agricultural lands. The importance of soil and plants in this pathway is the conversion of the metal in the soil to more soluble complexes that can be taken up and concentrated in different parts of the plant. When these plant components are consumed, the chemical form and amount of the metal in the plant complexes affect adsorption, distribution, and toxicity to target tissues and organ systems. Several programs of the Institute are concerned with metal toxicology and the physiological role of metallic elements.

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. Since 1980, exploratory discussions between both sides have been held during exchange visits with initial discussions centering around cooperation in the following areas: reproductive and developmental toxicology; validation of short-term test methods to detect and assess carcinogens, mutagens, and teratogens in the environment; 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-Finland Cooperation

A Memorandum of Understanding on Collaboration between NIEHS and the Finnish Institute of Occupational Health, Helsinki, was formalized in November, 1982. Areas of mutual interest include the following: pharmacokinetics; reproductive toxicology; neurobehavioral toxicology; genetic toxicology; epidemiology and risk assessment; and strategies for toxicological research priority settings. NIEHS and the Finnish Institute of Occupational Health co-sponsored an "International Workshop on Occupational Hazards Caused by Polychlorinated Biphenyls and Chlorobenzenes in Capacitors and Transformers", September 1983 in Finland. The proceedings of the workshop will be published in the NIEHS journal, Environmental Health Perspectives.

US-Italy Cooperation

Cooperation between American and Italian health scientists has taken place since 1977 under the Memorandum of Understanding between the U.S. Department of Health and Human Services and the Italian Ministry of Health.

Cooperation between NIEHS and Italian scientists has focused mainly on efforts to understand the mechanisms of toxicity associated with exposure to 2,3,7,8 tetrachlorodibenzodioxin (TCDD). Of special interest is the accidental exposure of humans to dioxins resulting from the explosion of a chemical reactor in Seveso, Italy, in 1976. Shortly after this incident, an NIEHS expert in TCDD toxicity visited Italy to consult with the Italian Government and provided Italian scientists with information on TCDD toxicity. In 1983, exchange visits took place to discuss potential future collaboration in the following areas: (1) Studies on the bio-availability of TCDD to organisms after the contaminant has been in soil for some period of time. The bioavailability of TCDD may well be a function of the type of soil, and experimental data are necessary in order to make better scientific risk assessments concerning the effects of accidental exposure to TCDD in the U.S. and Italian populations. (2) Studies on the chemical contamination of drinking water. Contamination of drinking water, particularly by halogenated hydrocarbons has become a worldwide problem. Of particular interest to both U.S. and Italian scientists is contamination of drinking water by trichloroethylene. Informal information exchange has also taken place in the areas of mutagenicity testing, long-term bioassay testing, testing of complex mixtures, and quantitative risk assessment.

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. In 1983, a joint U.S.-Japan Workshop on "Population Monitoring: Methods and Applications" was held in Honolulu, Hawaii. 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. Since that time, several exchange visits have taken place to discuss and exchange scientific information on these areas of mutual interest.

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. 1983 was the eleventh year of formal collaboration in environmental health research between the U.S. and U.S.S.R. Cooperative research efforts have 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, 1981, 1982, and 1983, 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; Kiev, May 1981, and Research Triangle Park, 1982 and 1983).

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 1983, exchange visits under this Agreement were conducted in research areas concerned with the mutagenic effects of environmental contaminants.

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 National 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 evaluation 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 1983 the Agreement was extended for another three years. In order to assist NIEHS participation

in the IPCS, a WHO Inter-regional Research Unit was established at NIEHS in 1981. Since the inception of the Programme, numerous scientific experts from NIEHS have participated on IPCS committees, special consultations, conferences, and technical working groups.

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 specialties necessary for the achievement of program goals. The IPCS provides an effective, multilateral mechanism for dealing with global environmental health issues and avoiding costly duplication of national efforts to test and assess chemicals.

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 (CCERP). 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) 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 provide information and advice on health hazards resulting from chemicals released into the environment and from 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 established a Hazardous Waste Information Evaluation Subcommittee (HWIES) which is chaired by the Assistant to the Director for International Affairs. The HWIES, composed of technical experts from various DHHS agencies, evaluates the available information on a number of chemicals frequently found in waste dumps and makes recommendations concerning the testing of these chemicals by the National Toxicology Program, and structured data record creation by the National Library of Medicine.

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

Task Force III (Ms. Hoffman, Mr. Kingman)

In the 1983 House Appropriations Report, NIEHS was directed to develop a new Task Force to review progress in selected research areas in the environmental health sciences. The results of this Congressionally mandated effort should provide a guide for NIEHS research through the 1980's. OPPE has been extensively involved in providing staff support for planning of the Task Force. Responsibilities have included organizing and coordinating activities of the Task Force Planning Committee. Through their recommendations regarding appropriate topics and participants, the Committee played a vital role in setting the framework for the Task Force. OPPE has also developed a detailed proposal which lays out the background and goals of the Task Force and suggests an organizational structure and time table of events. As the fiscal year came to a close, the Advisory Council considered the grant application for the support of the Task Force.

Superfund (Mr. Kingman, Ms. Hoffman)

OPPE is continuing its role as NTP liaison with the Centers for Disease Control (CDC) for Superfund activities. The office has been involved in various activities in support of NTP staff. OPPE completed negotiation over the terms of NTP's interagency agreement with CDC for the testing of chemicals found in waste dumps. A budget request for NTP FY 1985 Superfund activities has been submitted and approved by ASH, OS, and EPA. Finally, OPPE has been involved in working with CDC as that agency negotiated terms of its agreement with EPA.

Health Promotion and Disease Prevention (Ms. Hudson)

Throughout the year activity on the DHHS-wide program in health promotion and disease prevention continued. As lead agency contact for the Toxic

Agent and Radiation Control Implementation Plan, OPPE staff represented NIEHS and NTP, as well as the other DHHS cooperating agencies (i.e., FDA/NCDRH, CDC, NLM, and HRSA), at OASH meetings on prevention objectives and their implementation. During December 1982, OPPE convened a meeting of the Implementation Plan Work Group to review progress to date and to begin thinking about the progress review session to be held with the Assistant Secretary for Health. The Toxic Agent and Radiation Control Progress Review is scheduled for October 12, 1983; and recommendations from this review will be used in setting priorities for the agencies' efforts in toxic agent and radiation control. A summary of the progress review will be developed by OPPE staff in cooperation with staff from the other PHS Agencies for publication in Public Health Reports. OPPE staff also are ensuring that coordination takes place between DHHS participating agencies and the National Center for Health Statistics in developing portions of the Prevention Profile and the Health Interview Survey relating to toxic agent and radiation control.

During the past year OPPE staff has continued to give support to the NIEHS Deputy Director as he carries out his responsibility for providing oversight and coordination of the NIEHS disease prevention and health promotion program.

Dioxin/Agent Orange (Ms. Hudson, Ms. Miller)

As in previous years, the Director and staff of NTP had significant involvement in the Department's research on evaluation of the health hazards associated with human exposure to dioxins and Agent Orange. In support of this effort, OPPE staff provided a number of support services. OPPE staff also attended hearings held by the Senate Veterans' Affairs Committee on the scientific status of Agent Orange research, and tracked the progress of more than 12 legislative proposals dealing with compensation of Vietnam veterans exposed to Agent Orange and subsequently experiencing adverse health effects.

Asbestos (Ms. Hoffman)

Currently the Department of Health and Human Services (DHHS) is involved in litigation concerning Federal responsibility for health consequences of asbestos exposure in a number of cases. OPPE has frequently been asked by the DHHS Office of General Counsel (OGC) to assist in their preparation for various court cases. This has involved, among other things, response to requests for information and materials under the rules of discovery, the development of a deposition on significant asbestos research findings and preparation of budgetary information on NIH asbestos related research.

The Supreme Court recently ruled that only Federal employees and their families were barred from suing the government, not the defendants sued by the employees. Previously the Federal Employees Compensation Act had been interpreted as prohibiting defendants sued by Federal employees or their families from suing the Federal Government. OGC believes this ruling will substantially increase the number of suits involving DHHS. Because of this, OPPE expects their involvement in asbestos litigation issues will probably increase.

OPPE has also coordinated the preparation of testimony by Dr. Brandt, Assistant Secretary for Health, before a House Subcommittee interested in HHS asbestos related activities.

National Toxicology Program (Ms. Hudson)

As part of its continuing staff support to the NTP Director, OPPE staff carried out a variety of activities. Among these were development of answers to questions asked the Director at the Senate Appropriations Committee hearings; review of the NIEHS budget justification relating to the NTP; review of the FY 1984 appropriations hearings record on the National Toxicology Program; and revision of material on the NTP for EPA's "Federal Activities in Toxic Substances" Directory.

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 usually 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' 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.

Program Evaluation Activities (Ms. Hudson)

OPPE continued its involvement in a broad array of program evaluation undertakings through coordinating activities funded through the DHHS set-aside evaluation program and preparing the annual NIEHS Evaluation Plan.

During FY 1983, the evaluation of the NIEHS Environmental Toxicology Training Program, which was funded with set-aside monies and which OPPE staff helped develop, was completed; and it revealed that NIEHS supports approximately half of the existing training programs in environmental toxicology (State and institutional resources are the predominant source of support for the remaining programs). In addition, 30 percent of program directors for environmental toxicology training programs indicate their programs would stop if NIEHS training grant support were discontinued.

With regard to manpower supply/demand, the study found that although the number of job openings reported by employers during the 1978-1979 period was greater than the number of environmental toxicology trainees who had completed training--and a similar situation was projected by employers for 1982, fewer job openings than trainees were forecast for 1984.

To determine the validity of these projections, OPPE staff plan to coordinate development and conduct of follow-up surveys. NIEHS Extramural Program staff also are carrying out new activities as a result of this

evaluation project's findings. Because the study revealed that employers believe trainees to be inadequately trained in writing reports, computer use, and statistical bioassay, these staff currently are encouraging program directors of NIEHS-supported training programs in environmental toxicology to emphasize these technical skills in addition to the analytical skills traditionally taught in toxicology training programs.

During the latter part of FY 1983, OPPE staff assisted Extramural Program staff in developing the work scope and RFP so that a contract could be awarded for evaluating the NIEHS Environmental Epidemiology Training Program. This contract will be supported through set-aside funds; and it represents the second phase of the evaluation of the NIEHS training programs. Once completed, this project will assist in filling out the details of the manpower supply/demand situation for the environmental health sciences field.

Two evaluation projects described in the NIEHS FY 1984 Evaluation Plan are being funded with Institute resources; they are the Third Task Force on Research Planning in Environmental Health Sciences and the NTP Panel on Chemical Carcinogenesis Testing and Evaluation. OPPE staff is involved in only the first of these.

Budget Activities (Ms. Hoffman, Mr. Kingman, Mrs. Hudson, Ms. MacGregor)

OPPE frequently works with the Budget Office in developing a variety of materials. Most recently, OPPE staff participated in formulating the FY 1985 preliminary budget submission. Other involvement has included coordination and/or review of responses to Congressional appropriation questions, and the NIEHS budget justification.

OPPE had lead responsibility this year for developing the OMB required distribution of Institute research obligations according to whether they funded basic, applied or developmental efforts.

OPPE is conducting an analysis of NIEHS direct and indirect costs. Working with budget and program administrative staff, OPPE is looking at how indirect costs are currently spread within program areas and what the problem areas are in order to determine how a consistent rationale for identifying and calculating such costs can be developed.

Legislative Analysis Activities (Ms. Hudson, Ms. Miller)

As in the past, OPPE staff continued to maintain a legislative library of pending legislation and relevant background material on a wide range of issues. This baseline legislative effort supports specific Institute legislative analysis activities and is designed to keep the Institute Director and staff informed of legislative developments in Congress, to identify areas that may require Institute action, and to provide support for the Director's activities.

The development of public policy through the legislative process is one of the key areas of interest to OPPE, as well as to Institute staff. For this reason, OPPE staff monitored several issues of special interest, including

Agent Orange, victim compensation, and use of animals in research; and prepared information memos for distribution to affected or interested staff. OPPE staff also attended Congressional hearings on issues of importance to the Institute and prepared reports or analyses of the proceedings, which then were provided to appropriate NIEHS and NIH staff.

For issues of special interest, OPPE staff developed issue briefs and position papers for distribution to the NIEHS Director, OD staff, NIH Division of Legislative Analysis, and others in the Department and Congress as deemed appropriate. During the past year, OPPE staff worked with Institute scientists and administrative staff to develop a paper dealing with animal welfare in general and NIEHS efforts to reduce the use of animals through better test design and by developing alternatives to animal tests. This paper is intended to serve as background and briefing material for use by Institute and NIH staff and, if necessary, for developing required Congressional testimony on the subject.

Program Analysis Activities (Ms. Hudson, Ms. Hoffman)

Throughout the year OPPE staff were called upon to plan and carry out a variety of ad hoc program analysis assignments. Among these were:

- Identification of NIEHS research activities to be included in the NICHHD Maternal and Child Health Inventory for the NIH Director and for possible use at Congressional hearings.

- Identification and characterization of NIEHS research related to endocrinology and metabolic diseases for the NIADDK report to the Senate Appropriations Committee.

- Survey of NIEHS research related to teratogenesis for the NIH Director's use at Congressional hearings.

- Development of a summary of NIEHS-supported research related to autoimmune deficiency syndrome (AIDS) to revise an AIDS Chronology maintained for the use of the NIH Director by the Division of Legislative Analysis.

- Preparation of numerous reports on NIEHS research related to ocean pollution for the NOAA Annual Report to Congress, the OMB Health Branch, the Annual Update of the Catalog of Federal Assistance, and the NOAA Office of Policy and Planning.

- Development of briefing materials and discussion format for a meeting between NIEHS Director and Congressman Valentine (representing the District in which NIEHS is located) and his staff.

- Detailed review and comment on the section of the President's Private Sector Survey on Cost Control dealing with toxicology consolidation in the Public Health Service.

- Coordination of responses to House Subcommittee questions on pesticides and the Administration's cancer policy.

- Provision of information and material on asbestos health hazards to the Congressional Research Service.

Administrative Activities

NIH is continuing efforts to develop appropriate classification guidelines for the program analysis series, with little apparent progress. In the interim, following the rewriting of the OPPE Program analysis positions, NIEHS asked the Personnel Office to review the classification of the professional positions in OPPE with a view toward determining their appropriate classification. This will be the first formal internal review of the positions in OPPE since it was established in 1977, with the exception of the position of the Director, OPPE which was reviewed by OPM staff during their recent review of the Institute. It is hoped that this review will result in more appropriate classification of the professional staff positions in the office.

This year saw the broad scale introduction of the word processor in the Institute. This has been of enormous assistance to the OPPE, although at this time OPPE has not yet been assigned a machine with enough capabilities to carry its typing load adequately. In addition OPPE requires a CPT with communicating ability. Many of OPPE's final products need to be transmitted to geographically remote offices in Washington as well as Atlanta. Having communicating ability at hand would greatly simplify this task.

Since last years report, Dorothy Williams joined the staff as clerk-typist and has since transferred to the Budget Office. She is missed; however, OPPE was lucky in being able quickly to find a replacement who will report for duty before the end of the fiscal year. Throughout this year, the OPPE has received strong support from Ms. Hunt, the secretary to the Office Director. Somehow in the face of an ever increasing workload, she and her supporting staff have managed to produce the OPPE work products, and an untold number of briefing books, as well as to manage the office and insure official travel was accomplished without realizing its potential for confusion. Early in the fiscal year, Ms. Hoffman was detailed to the Director's Office for approximately two months to assist in coordinating plans for the formal dedication of NIEHS's permanent facility.

As the year came to an end, Ms. Gay MacGregor accepted a Presidential Management Intern position with the Environmental Protection Agency in Washington, D.C.

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 201,665 square feet of leased space and 464,868 square feet in government-owned facilities on the South Campus site. The North Campus site provides 154,274 square feet in a complex of 19 buildings, with a staff housing capability in excess of 500 permanent full-time employees.

The new NIEHS laboratory, administrative and support facilities were completed and are on a 509-acre tract of land in the Research Triangle Park that is in close proximity to the North Campus facilities. It is expected that the new facility will have been fully occupied by close of FY 83. 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. 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 Trailer City site was "fine tuned" and currently provides office space for the Extramural Program.

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	
Warehouse:		
Old Raleigh Road	15,500	
Asbestos Storage	6,166	
Neptune Storage	5,700	
C&O	2,500	
Duke Animal Colony Bldg	6,500	
SUBTOTAL	47,391	13,248
TOTAL	201,665	464,868

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 1983, 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. Normal staff turnover presented problems for the OFE because of the DHHS wide freeze on employment, and the need to request certificates of eligibility from Raleigh. Recruitment activities continue in order to fill the 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 were confined to those changes which were 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 reinterate, in FY 1983, 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 continuing its efforts to see certain NIEHS laboratories relocated into the permanent facility on South Campus and renovations begin on North Campus. Areas of interest are: (a) energy conservation, and (b) phased implementation of a preventive maintenance program for both building service and biomedical laboratory equipment. 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.

HEALTH AND SAFETY

HEALTH AND SAFETY OFFICE
Summary Statement

The NIEHS Health and Safety Office is administratively located within the Office of the Deputy Director and has broad responsibility for chemical and radiation safety, physical safety, fire protection, emergency preparedness, environmental protection and occupational health surveillance. In FY83 the program staff was increased by addition of a fire protection specialist and a technician to manage Institute special containment facilities on a daily basis. The health and safety program also relocated to its expanded office and laboratory areas in the NIEHS permanent facility.

Toxicological research laboratories pose unique health and safety problems due to the great variety of chemicals and radioisotopes that may be used and the non-routine nature of many laboratory procedures. The primary emphasis of the NIEHS Health and Safety Office is to minimize exposures through utilization of containment equipment, following appropriate work practices and procedures and use of personal protective equipment. The primary tool for accomplishing this objective is the required hazardous agent safety protocol and employee training. During FY83, a computer record system for hazardous agent protocols was fully implemented significantly improving responsiveness to questions and emergencies such as chemical spills.

Research at NIEHS involves a wide variety of potentially hazardous chemical agents. Programs for safe use of these substances remain high priority. During FY83 the Institute's capability for industrial hygiene exposure monitoring was significantly improved by establishing an industrial hygiene laboratory with emphasis on direct reading instrumentation. In addition to routine quarterly surveys of Institute laboratories and other facilities, special exposure monitoring studies were completed for noise, formaldehyde, anesthetic agents, organic solvents and asbestos. As a result of these studies, improved techniques for administering anesthetic gases to small laboratory animals were developed. To reduce formaldehyde exposures in necropsy areas, a free standing hood was designed and fabricated.

Special problems associated with administering test compounds to laboratory animals received added attention in FY83. Jointly, the Health and Safety Office and the Comparative Medicine Branch developed a policy for required containment facilities for dosing studies involving hazardous chemicals and radioisotopes. This policy serves as a guide to researchers in planning their hazardous agent safety protocols. Ventilated cage rack units were purchased to allow toxic substance animal studies to be conducted in animal rooms served by the clean-dirty corridor principle. A special research effort was undertaken to rigorously evaluate performance of the ventilated cage rack as a primary containment device.

While proper containment and appropriate work practices are the primary means of minimizing employee exposures at NIEHS, personal protective equipment is often used for absolute assurance. The Institute's respiratory protection program was formally defined and upgraded in FY83.

Emphasis is placed on appropriate respirator selection and qualitative fit testing.

NIEHS is moving quickly toward office automation with increased use of video display terminals. In response to these changes, a safety and health program for video display terminal operators was developed and fully implemented in FY83. Key elements of this program are provisions for appropriate ergonomically designed work stations, proper illumination and glare control and appropriate work-rest regimens.

Expansion of the NIEHS scientific staff and availability of additional laboratory space have substantially increased the Institute's use of radioisotopes and radiation sources. 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 biological monitoring program was substantially increased in FY83 to include routine urine monitoring of ^{14}C and ^{3}H users. A special study to monitor stack emissions during incineration of liquid scintillation vials was completed in FY83.

There were several noteworthy accomplishments in the Institute's hazardous waste management program during FY83. The Institute's program was found to be in full compliance with all provisions of RCRA during the interim status inspection by the State of North Carolina. Facilities for packing and temporary storage of hazardous wastes were vastly improved through occupancy of appropriately designed chemical storage facilities. The Institute's ability to incinerate hazardous wastes was upgraded by modification of an existing pathological incineration for higher temperature operation with increased residence time for greater destruction efficiency. Liquid scintillation vials and flammable wastes are now incinerated resulting in substantial cost savings while at the same time using an environmentally sound treatment technology. Finally, a computer system for maintaining records of hazardous waste disposal activities was developed and fully implemented. A collaborative effort with the NIH Division of Safety was initiated to investigate alternate treatment methods for laboratory wastes.

An important component of the NIEHS health and safety program is the Institute's occupational medicine program. During FY83 basic medical services as well as periodic surveillance programs were integrated to improve efficiency. The Occupational Medicine Clinic planned for the NIEHS permanent facilities was equipped as necessary and a scope of work negotiated with the PHS, Division of Federal Employee Occupational Health. This clinic will be operational by the first quarter of FY84. Various automated occupational health and safety record systems for the NIEHS clinic were investigated.

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 FY83 the Health and Safety Office conducted four sessions of the General Laboratory Safety and the Radiation Safety courses. One hundred eighteen (118) employees attended the General Laboratory Safety Course and 96 attended the Radiation Safety Course.

Emergency preparedness received considerable attention during FY83. Occupant Emergency Plans were implemented 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 FY83 training sessions concerning selection and use of fire extinguishers were conducted.

The health and safety program devoted a large amount of time to shake-down and planning for occupancy of the permanent laboratory facilities. These tasks included evaluation and certification of containment equipment such as fume hoods, biological safety cabinets and filter units, fire inspections and testing of fire safety systems, monitoring of drinking water quality, sound level measurements, evaluation of emergency equipment such as eyewashes and safety showers, certification of laboratory gas systems and evaluation of HVAC systems. The Health and Safety Office also assumed responsibility for managing all high hazard laboratories. Equipment for these rooms was purchased and standard operating procedures developed.

LIBRARY AND INFORMATION SERVICES OFFICE
Summary Statement

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include reference services, computerized literature searching of bibliographic and scientific data bases, maintenance of a collection of 700 periodical titles and 10,000 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 1,673 new books for the Library and the laboratories, and publication of a monthly newsletter and the annual bibliography of publications by NIEHS personnel.

Reference/Literature Searching: The Library maintains one of the most advanced computerized literature searching capabilities in the world, with access to more than 200 data bases covering subjects from toxicology to business administration. During FY83, Library personnel performed comprehensive multi-data base searches on over 1,000 topics and answered over 1,500 reference questions. The addition of a new reference librarian allowed more in-depth searches to be carried out. The most heavily used data bases continued to be TOXLINE, MEDLINE, Toxicology Data Bank, Biological Abstracts, and Chemical Abstracts. The Library served as a test search center for the Chemical Carcinogenesis Research Information System and the Dermal Absorption Data Base as well as for Hazardline, a new toxicity and chemical exposure file.

Journal Collection: The journal literature continues to be the primary means of disseminating scientific information. The Library subscribed to approximately 700 periodicals during FY83, and, in addition, ordered 362 subscriptions for the various laboratories. Access to the subscriptions contractor's data base via a computer terminal in the Library expedited journal check-in, claiming, and ordering. The Library continued to selectively bind journals or replace them with microfilm to save space. Some older volumes were put in storage to relieve the overflowing shelves. The collection now includes 11,600 journal volumes and 1,300 microfilm reels. The Library, working with the EPA Library, updated its computer-generated journal holdings list for 1983.

Book Collection: Continuing the development of the book collection, the Library ordered 1,673 books in FY83, of which 34% were ordered for the Library and 66% for the laboratories. The 570 books ordered for the Library represented a 22% decline over FY83, a reflection of budget constraints. The Library also ordered more than 200 technical reports.

Culminating several years of planning, the Library installed the C.L. Systems LIBS 100 computerized catalog and circulation system. This computer supports an online catalog of the books in the Library and is searchable by author, title, or subject using terminals in the Library or from any terminal in the labs or offices, thus making it much easier for Institute scientists to find out what books are in the Library. The system also speeds up the check-out procedure, produces overdue notices, and provides statistical reports for management purposes. Since its installation in January 1983, the system has been used to check-out 235 books per month. In addition to designing the data base and training the staff, much time was devoted to demonstrating the system to Institute personnel and to libraries from around the state.

The Library continued using the automated cataloging system, OCLC, a computerized union catalog of books held by more than 3,500 libraries nationwide. 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. Through an interface, catalog records are transmitted from the OCLC computer in Ohio to the LIBS 100 computer in the NIEHS Library where they are immediately integrated into the public catalog.

Interlibrary Loan: The number of photocopy and loan requests did not change substantially in FY83, the total being 12,647. However, for the first time, more of the requests were filled from the Library collection (52%) than from other libraries through interlibrary loan (48%). This reflects the ongoing improvement in the NIEHS collection.

The OCLC computerized catalog also proved useful for verifying titles for interlibrary loan and for locating libraries from which to borrow books throughout the U.S. The NIEHS Library provided 275 loans or photocopies to other libraries as well.

Institute Manuscripts and Bibliography: The Library continued to maintain the NIEHS archives of manuscripts submitted for publication and list them in the monthly newsletter. The Library published the 1982 NIEHS Bibliography, a catalog of the papers published by Institute personnel since 1966.

Experimental Data Repository: The Library was assigned the responsibility for archiving primary data generated at the Institute. A system was set up for microfilming laboratory notebooks and preliminary plans were made for storing other experimental media.

Planning and Meetings: Plans for library and information services for personnel in the new South Campus laboratories began being implemented in FY83 using three reading rooms in the E-module. Arrangements were made with the scientists in adjacent offices to house some personal and some Institute-owned journals there. In Building 18, Library staff offices were moved out of the Library itself and down the hall, providing more critically needed space for books and journals. These measures have temporarily solved the space problem but will last only a year or two.

Close contact with various library and information organizations was maintained in FY83. Dav Robertson served as Chairman of the Special Libraries Association's Environmental Information Division and planned and presided over programs on hazardous waste information resources and environmental regulation resources at the annual conference. He also served on the N.C. Advisory Council to the State Library for dispersal of Federal funds to libraries. Ralph Hester represented NIEHS at the annual meeting of the Bibliographic Retrieval Systems User Group, and Christine Chastain represented NIEHS at the annual meeting of the Medical Libraries Association. The Library hosted the N.C. Special Libraries Association conference on access to government information.

INTRAMURAL RESEARCH PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR

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, neuropeptides and neurophysiology.

NEUROBEHAVIORAL TOXICOLOGY

The research of this group is devoted to characterizing the effects of environmental factors on behavioral and neurological function and elucidating their behavioral mechanisms of action. Also of interest are studies that investigate the conditions which predispose individuals to neurotoxicity with a special emphasis on the developing animal.

The primary research objective of the Neurobehavioral Workgroup has been to develop an animal model for the neurotoxicity produced by organochlorine-like insecticides, such as chlordecone. Time- and dose-response functions for the main neurobehavioral effects of chlordecone (i.e., tremor, hyperexcitability) have been established in adult rats. Similar experiments on related organochlorines (i.e., lindane, DDT) are currently underway. Thus far, the research suggests that pontomedullary nuclei in the brain stem are activated by chlordecone to produce exaggerated responsiveness to novel stimulation (hyperexcitability) and tremor. Structures below the brain stem (e.g., spinal cord) appear to be necessary for the expression of these effects, while the role of higher brain centers (i.e., cerebellum, corpous striatum, motor cortex) remains uncertain. What is clear is that higher brain centers modulate the expression of chlordecone-induced neurotoxicity. The mechanism by which chlordecone produces hyperexcitability and tremor is not known, although it seems likely that this agent interferes with crucial neurochemical steps, perhaps ionic exchange during the propagation of nerve activity, to produce increased membrane excitability. Whether or not this is a common feature of organochlorine insecticides is not known.

In addition to its direct effects on the nervous system, chlordecone may alter behavior by affecting the integrity of the hypothalamic-pituitary-adrenal (HPA) axis. Rats exposed to chlordecone during postnatal development display the usual signs of acute chlordecone neurotoxicity. However, subsequent behavioral testing has revealed that animals exposed to chlordecone during the postnatal phase of neuroendocrine differentiation display long term changes in behavioral reactivity which appear to be associated with chlordecone-induced alterations in basal adrenal cortical function. The hypothesis that chlordecone interferes with the ontogeny of the HPA axis is currently being explored in both rats and in an avian (Japanese quail) species. The role of neuroendocrine factors in the expression of chlordecone-induced neurotoxicity in animals exposed as adults remain to be elucidated.

The Neurobehavioral Workgroup is also characterizing the neurobehavioral toxicity of representative organometals, such as triethyl and trimethyl tin and lead. Previous research has indicated that triethyl tin produces a generalized suppressant effect on behavior, which is associated with edema in white matter in the nervous system. Reports from other laboratories have indicated that some organometals such as trimethyl tin produces selective degeneration of pyramidal cell fields in the CA3 - CA4 region of the hippocampus. Such neuropathological effects have specific neurobehavioral consequences (i.e., changes in responsiveness to noxious and nonnoxious stimuli, deficits in short-term memory) that are being characterized by the Neurobehavioral group. The hypothesis that the limbic system is a vulnerable site for the expression of neurotoxicity of some organometals is also being investigated in developing animals. A single injection of triethyl lead at a time postnatally when the hippocampus is still being formed in the rat results in the predicted neuropathological effects (i.e., loss of specific pyramidal cells in the hippocampus) and associated alterations in neurobehavioral function. The mechanism by which certain organometals selectively affect the limbic forebrain is currently under investigation.

NEUROPHARMACOLOGY

The primary goal of the Neuropharmacology Workgroup is concerned with the neurochemical and neuropharmacological bases for the effects of neurotoxicants on behavioral and neurological function. The major research effort has focused on the chemical modulation of neurotransmitters and neuropeptides. The major neurotoxicant studied is chlordecone. The purpose of our approach was to examine a possible involvement of either neurotransmitters or neuropeptides in chlordecone-induced tremor or neuroendocrine dysfunction.

A series of methods employing high performance liquid chromatography have been developed for routine measurement of neurotransmitters and their major metabolites. A variety of radioimmunoassay methods have also been developed for the analysis of levels of peptides and hormones in the brain or tissue fluids. An *in vitro* cell-free translation method has been used for the measurement of biosynthesis of enkephalin in the brain.

The study concerning the possible mechanism underlying chlordecone-induced tremor has suggested that this organochlorine insecticide may increase neuronal excitability by acting on sodium channel which may be responsible for the increase in the turnover of 5-HT and acetylcholine. In turn, the increase in the turnover of these two neurotransmitters may contribute to the expression of chlordecone-induced tremor.

Chlordecone exerts robust effects on the hypothalame-pituitary function such as the pituitary enkephalin system. Our study has shown that the estrogenic activity of chlordecone may be related to its effect on this peptide system. Further, we have shown that the estrogenicity of this insecticide may be responsible for its effects on the plasma level of LH and prolactin. This study provides some neurochemical basis for chlordecone-elicited toxicity in neuroendocrine functions, such as reproductive failure.

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.

The neurotoxicity of organic lead compounds has been the object of major study this last year. The hypothesis has been tested, that this class of compounds owes some of its toxicity to the ability of lead to compete with other divalent metals, including calcium, zinc, copper and selenium. The levels of several enzymes dependent on these cation, in brain tissues of adult male rats treated with 7.9 mg/kg triethyl lead chloride (TEL), by subcutaneous injection, have been examined. This dose of TEL is 75% of the LD_{50}.

Levels of phosphodiesterase, a calcium requiring enzyme, were unchanged at 1, 7, 14 and 28 days after TEL-treatment. The brain region chosen for these studies was the hippocampus as this area is known to be selectively sensitive to damage by organic lead and tin compounds. The calcium channel within hippocampus was assayed using a radioactive calcium channel antagonist (nitrendipine). This was also unaltered at the experimental intervals described. High affinity binding of labeled calcium to hippocampal membrane was not modified by TEL-treatment. In vitro study of this interaction shown both TEL and inorganic lead to be rather poor competitors for calcium binding sites.

The zinc containing enzymes leucine aminopeptidase and glutamate dehydrogenase were not depressed in TEL-treated rats but hippocampal superoxide dismutase (a zinc and copper requiring enzyme) was transiently elevated in rats dosed with TEL. This implied a protective response to hyperoxidative conditions rather than a displacement of zinc by TEL. This effect had some regional specificity since it was much less pronounced in the frontal cortex. However, assays of rates of lipid peroxidation suggested frontal cortical damage was caused by TEL-exposure. Glutamate dehydrogenase was depressed 28 days after dosing, in the hippocampus. This rather belated change may be secondary rather than related to zinc definciency. The sodium pump, within cerebral membranes is magnesium requiring and was as prevelant on hippocampal membranes from TEL treated rats as on controls, as judged by ovabain binding.

Neurotransmitter and related receptor binding assays have also been carried out on membranes from treated rats. At 28 days after TEL-injection both muscarinic cholinergic and GABA receptor binding were elevated in the hippocampus but depressed in the cortex, suggesting a reciprocal adaptive response. Benzodiazepine receptors were transiently depressed in the hippocampus of treated rats. The time course of this change may correlate with the temporary analgesia found in TEL treated rats.

Other work has been performed with rats exposed to parallel doses of organic tin compounds. Trimethyl tin appears to alter cerlinal glucose consumption in a bimodal manner, initially depressing this parameter but later causing a significant elevation of cerebral glucose utilization. This effect is not confined to a single region but occurs in hippocampus, cortex and striatum and is not associated with any significant changes in body or brain region weights. Body temperature was also unchanged by treatment. There may be a relation between the hyperreactivity of tin-treated animals which has a gradual onset, and cerebral glucose consumption.

Neonatal treatment of rats with triethyl tin severely affects the rates of cell proliferation in hippocampus, posterior cortex and cerebellum. These sensitive regions undergo considerable postnatal neurogenesis. Cells thus become more densely packed. The RNA content per cell is unchanged in these affected regions but cells have a greater concentration of protein. This may be due to the presence of reactive astrocytes since these are relatively small and dense. Some degree of sparing of cholinergic neurons was suggested by acetyl cholinesterase levels of triethyl tin-treated rats.

PEPTIDE NEUROCHEMISTRY

The Peptide Neurochemistry Workgroup joined the Laboratory October 1982, following an administrative reorganization of intramural programs.

This workgroup was previously part of the Laboratory of Pulmonary Function and Toxicology (LPFT) and had focused on the presence of peptides in neuroendocrine cells and in a human lung tumor, the highly metastatic small-cell carcinoma which was grown in nude mice. The particular peptides they chose to investigate belong to 2 classes of amphibian peptides, the tachykinins or physalaemin-related peptides of which substance P is a member, and the bombesin peptides. Their approach was basically biochemical and immunological in nature. They developed highly specific immunoassays for bombesin and physalaemin that did not cross-react with substance P or any other known peptide. Using these antisera, they were able to demonstrate that this human lung tumor contained immunoreactive material that closely resembled the amphibian peptides, bombesin and physalaemin. The significance of this work is underscored by the fact that bombesin is present in all cell lines derived from this lung neoplasm and is currently being investigated as a possible peptide marker for the clinical assessment of the progression of this disease.

The other main area under investigation which is quite interesting is the presence of bombesin in milk. From their familiarity with the physiological and pharmacological properties of bombesin, they searched for bombesin immunoreactivity in other mammalian tissues and fluids besides the tumor. Historically it was known that extraneously administered amphibian bombesin brings about an increase in the plasma levels of many gastrointestinal hormones as well as gastric acid in the stomach. These data coincided with other well known data, namely, that the peroral consumption of milk in infants also leads to rises in virtually the same hormones and also gastric acid. Thus, they assayed bovine milk. Chromatographic and preliminary chemical analyses has provided evidence that this milk "bombesin," is larger than the 27 amino acid gastrin-releasing peptide, called GRP, which is the mammalian form of bombesin. These findings are of particular interest due to the medical profession's recent reversal of

the use of milk in ulcer therapy. It should be noted that an 8 ounce glass of milk contains over 200 nanograms of bombesin-equivalents which is more than enough to increase gastric acid production in an adult. An extension of this study is the direct application of their radioimmunoassay on human breast milk. They find bombesin levels ranging from approximately zero to over 650 picograms for a milliliter of milk, which appears to depend on the time of delivery.

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.

Some members of this workgroup have long been interested in the electrophysiological basis of cochlear transduction and the elucidation of physiological mechanisms which lead to damage produced by noise and/or chemical agents. In particular, they are concerned with the permeability to ions and water of the cochlear partition which is directly linked to the mechanoelectric transduction process. They have found that cochlear function is dependent on membrane permeability to potassium ions but is relative independent of changes in sodium ions permeability. Both noise and the ototoxic antibiotic, kanamycin decrease potassium conductance of the organ of Corti.

Other members of the Neurophysiology Workgroup are investigating the neurophysiological effects of organometals. Although the signs and symptoms of organometal toxicity in humans are well known, the precise sites and mechanisms of action are poorly understood. Work in this and other laboratories have provided evidence that the limbic system is a vulnerable site for effects of at least some organometal compounds; particularly trimethyl lead, triethyl lead and trimethyl tin. Studies are therefore in progress to determine the anatomical sites within the limbic system upon which these agents act and to confirm or refute the hypothesis that the limbic system is the most vulnerable area in the brain for perturbation by these compounds.

NON-IONIZING RADIATION

The non-ionizing Radiation Workgroup has studied the biological effects of 2.45 GHz microwave radiation during the past year. Both *in vitro* and whole animal biological systems have been utilized in order to study both the basic interaction mechanisms of microwaves with biological material and the overall effects on more complex specimens - the whole animal. *In vitro* preparations used have included microtubules from calves brains, rat atria, and frog sciatic nerves. No effects of microwave radiation were observed on microtubular

polymerization or chronotropic and inotropic changes in spontaneously beating rat atria. From these negative results important information can be extracted and used to assist in explaining information reported in the literature. No effects of microwave radiation on microtubular polymerization is an important result because of the association between the dynamics of microtubule formation and a variety of biological processes such as cell division, brain development, behavior, and teratological aberrations - all of which have been reported to be influenced by microwave radiation. Therefore, a molecular basis for these reported effects of microwave radiation will probably be found in some other aspects of cellular physiology.

Isolated sciatic nerves of frogs have been exposed to both continuous wave (CW) and pulsed wave (PW) microwave radiation. The nerves in both cases were stimulated at a high rate (50 twin stimulating pulses per second) and the time required for the nerves to fatigue was observed. Both the CW and PW exposed nerves lost their vitality (the ability to sustain a high firing rate over a prolonged period) almost twice as fast as the unexposed nerves. In the PW exposures the asynchronization (the microwave pulses delivered at varying times in the firing cycle) or synchronization of the microwave pulses either during the peak of the nerve action potential or during the quiscent period between nerve firings produced the same effect. The slowness with which the microwave-induced loss of vitality develops, along with the irreversibility, indicates that the effect may result from an interference with long-term regulatory processes (such as interfering with maintenance of adequate ionic concentration gradient across the membrane) rather than an immediate interference with the mechanism for action potential firing.

In the past much of our research using whole animal systems was directed toward the study of embryofetal toxicity and gross teratogenicity (congenital malformations) due to microwave exposure. No effects were observed unless levels of exposure which produced significant heating were used. During the past year studies to determine the effects on the functioning organism as it matures of exposure during embryogenesis to microwave levels which do not increase temperature have been a major part of our research. Pregnant rats and fertilized Japanese quail eggs both have been used in these studies.

Pregnant Fisher rats were exposed 3 hours per day from days 5 to 20 of pregnancy to a microwave power density level of 10 mW/cm^2. One group of offspring received no additional exposure after birth while a second group was exposed from day 2 through 20 postnatally. A battery of neurobehavioral tests were performed on both groups of animals at 30 and 100 days of age and compared with a control group of animals which were treated the same except for radiation exposure. The 30-day-old animals which had the additional 20 days of exposure after birth had a significant reduction in swim time to exhaustion whereas there was no difference in the 100-day-old-animal. The experiment was repeated and again the 30-day-old animals which had the 20 days of exposure after birth had significantly shorter swim time to exhaustion.

Fertilized Japanese quail eggs were exposed to 2.45 GHz microwave radiation with an incident power density of 5 mW/cm^2 in order to investigate the effects of exposure during embryogenesis on humoral and cell-mediated immunity. The embryos were exposed for 24 hours per day for the first 12 days of development. At 6- and 12-weeks of age the immune responsiveness was determined. Humoral

immune responsiveness was assessed by immunizing quail with Chukar quail red blood cells. Hemagglutinin titers and relative levels of IgM and IgG were measured. No changes in humoral immune responsiveness were found in either age group. Cell-mediated immune potential was evaluated using the mitogen phytohemagglutinin (PHA-P) in saline which was injected in one wing web. An equal volume of saline was injected into the alternate wing web. The magnitude of the response (web index) was calculated as the ratio of the post-exposure to pre-exposure skin thickness. The web indices of the 6-week-old quail were not significantly different. However, web indices of both the 12-week-old male and female microwave-exposed quail were significantly less than their respective non-exposed strains. These results show that embryonic exposure to microwave radiation reduced cell mediated immune responsiveness in the adult Japanese quail but had no effect on humoral immune responsiveness. Further research using the Japanese quail as a test system will be conducted under a research support contract with North Carolina State University.

PERSONNEL

Additions to the Laboratory were: Acoustical Engineer - Dr. R. Cook; Senior Staff Fellow - Dr. M. Galvin; Visiting Fellow - Dr. T. Kanamatsu; Medical Officer - Dr. T. Konishi; Research Chemist - Dr. L. Lazarus; Research Physicist - Dr. D. McRee; Visiting Fellow - Dr. Y. Muratsuka. Individuals leaving the Laboratory of Behavioral and Neurological Toxicology were: Expert - Dr. L. Uphouse; Visiting Fellow - Dr. K. Yoshikawa.

OTHER ACTIVITIES

Dr. S. C. Bondy: Adjunct Associate Professor, Department of Pharmacology, University of North Carolina, School of Medicine; Member, NIEHS Radiation Safety Committee; Member, Publications and Education Committee, American Society for Neurochemistry; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial Board, International Journal of Developmental Neuroscience; Member, Editorial Board, Developmental Neuroscience; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurochemical Research; Member, Organizing Committee of the Winter Conference on Brain Research; Invited seminar "Biochemical Evaluation of Neurotoxicity," University of California, Irvine; Invited seminar "Detection of Damaged Neuronal Circuiting by Analysis of Neurotransmitter Receptors," University of Texas, San Antonio; Ad Hoc Reviewer, National Science Foundation (Neurobiology Program); Ad Hoc Reviewer, Petroleum Research Fund.

Dr. J.-S. Hong: Adjunct Associate Professor, Department of Psychiatry, Duke University Medical School; 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 Virginia, Richmond, VA; Invited seminar entitled "Regulation of Brain Enkephalins by Estrogen," EPA, Research Triangle Park, NC. Guest reviewer for Brain Research.

Dr. T. Konishi: Ad Hoc Reviewer for Journal of Acoustical Society of America; Ad Hoc Reviewer for Hearing Research; Ad Hoc Reviewer for National Science Foundation (Sensory Physiology and Perception); Invited participant, International Symposium on Hearing - Physiological Bases, Psychophysics and Behavioral Studies at Bad Neuheim, West Germany, April 5-9, 1983; Invited participant, 19th Workshop on Inner Ear Biology at Mainz, West Germany, September 5-8, 1982.

Dr. L. H. Lazarus: Adjunct Associate Professor, Department of Pharmacology, School of Medicine, University of North Carolina; Adjunct Member, Cancer Research Center, School of Medicine, University of North Carolina; Ad Hoc Reviewer for Analytical Biochemistry, Cancer Research, Chemico-Biological Interactions, Journal of Biological Chemistry, Proceedings of the National Academy of Sciences, and Science; Invited seminar entitled, "Amphibian Peptides in Health and Disease," University of North Carolina.

Dr. D. I. McRee: Adjunct Associate Professor, Department of Poultry Science, NCSU; Coordinator US-USSR Cooperative 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 Non-Ionizing 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 Non-Ionizing Radiation; Appointed to National Research Council Committee on Biological Effects of Non-Ionizing Radiation; Appointed member of IEEE's Committee on Man and Radiation (COMAR); Elected to membership on USA Commission A (Electromagnetic Metrology) of the International Union Radio Science; Appointed to American Technical Program Committee for Joint BEMS-LLRSI Open Symposium on Tlectromagnetic Fields and Biological Systems to be held in Florence, Italy, in August 1984; Appointed to N.C. Board of Science and Technology Committee on Scientific Equipment Requirements; Appointed to Editoral Review Board of Environmental Health Perspectives; Invited speaker to the N.C. Chapter of Health Physics - "Overview of Radiofrequency and Microwave Radiation"; Member, 9-11 Promotion Committee, National Institute of Environmental Health Sciences; Reviewer of manuscripts for Life Sciences, Radiation Research, Journal of Microwave Power, Differentiation, and Electromagnetics Journal; Reviewer of manuscripts and documents for EPA and the International Radiation Protection Association; Reviewer of NSF Grant Applications on Biological Effects of Electromagnetic Fields.

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; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology; Member, National Institute of Environmental Health Sciences Labor Management Committee; Member, 9 to 11 Promotion Committee, National Institute of Environmental Health Sciences; Chairman, Committee on Methods in Neurobehavioral Toxicology, International Programme on Chemical Safety, World Health Organization; Invited seminar entitled "Aspects of the Tremorigenic Properties of Chloredecone," Department of Pharmacology, University of 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; Associate Editor (Field Editor for Neurobehavioral Toxicology) for Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology and Teratology; Invited seminar entitled "Studies on the Mechanism of Chlordecone-Induced Tremor," Medical College of Georgia, Augusta; Consultant, National Center for Toxicological Research, Jefferson, AR; Consultant, Organization for Economic Cooperation and Development, Ad Hoc Expert Group on

Neurotoxicology; Consultant WHO International Program on Chemical Safety (IPCS), Working Group on "Methods for the Integrated Evaluation of Risks for Progeny Associated with Prenatal Exposure to Chemicals; Consultant, WHO IPCS Working Group on "Methods in Neurobehavioral Toxicology"; Consultant, Research Project of U.S. Army Medical Bioengineering Research and Development Laboratory; Program Chairman, Behavioral Teratology Society, 1983; Secretary, Behavioral Teratology Society; Member, Animal Care Committee, National Institute of Environmental Health Sciences; Ad Hoc Reviewer, Social and Behavioral Sciences Research Grants Program, March of Dimes; Ad Hoc Reviewer, National Science Foundation Psychobiology Program; Ad Hoc Reviewer, Environmental Protection Agency; Invited participant, US-USSR Workshop on Physical Factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25019-02 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physalaemin-like Peptide in Mammalian Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lawrence H. Lazarus Research Chemist LBNT NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Environmental Chemistry
University of North Carolina, Chapel Hill

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term objectives of this research project are to identify and characterize the mammalian form of the amphibian peptide physalaemin and to determine its physiological and pharmacological mode of action. The initial focus has been to develop a scheme to purify this immunoreactive substance from kilogram quantities of tissue. Dry rabbit stomachs, which contain the highest amount of the physalaemin-like immunoreactivity (PSLI), were first extracted in an ethanol-formic acid solution to remove interfering mucins. A concentrated and clarified extract was passed through cation and anion exchange resins, molecular sieving chromatography and absorbed on reverse-phase supports. The most purified preparation to date is a colorless solution or a white powder in the dry state, and a single absorbance peak can be attributed to an immunoassayable quantity. Final proof of its purity requires several more chemical analyses. PSLI differs from physalaemin, however, based on the following chemical traits: neutral, hydrophilic, not readily absorbed on glass, an apparant larger molecular weight, differentially cross-reacts with distinct physalaemin antisera, and contracts guinea-pig ileum about 20-25% of that observed with physalaemin. This hitherto unknown neuropeptide may thus prove to be a prototype of a new class of mammalian peptide hormones.

Principal Investigator and All Other Personnel Engaged on the Project:

L. H. Lazarus	Research Chemist	LBNT	NIEHS
W. E. Wilson	Research Chemist	LBNT	NIEHS
B. J. Irons	Biological Technician	LBNT	NIEHS
O. Hernadez	Research Chemist	LEC	NIEHS
D. Klapper	Assist. Prof.	UNC, Chapel Hill	
V. Erspamer	Professor	Univer. Rome, Italy	

PROJECT DESCRIPTION

METHODS EMPLOYED: Powdered, freeze-dried rabbit stomachs (100 gm lots) were extracted in 10 volumes 90% ethanol-1.2 N formic acid. The extract was clarified by centrifugation and the volume reduced in vacuo, re-extracted with water and the aquous phase passed through Dowex 50 and Dowex 1 resins. The eluates were absorbed on an octyl silyl packing material and removed with methonol solutions. This fraction was chromatographed on Bio-Gel P-4 and the peak fraction applied to an analytical HPLC column. Recycling this later step through P-4 and HPLC yielded a multi-peak absorbance pattern, one peak of which corresponded to immunoassayable material. Pharmacological monitoring of bioactivity used guinea pig ileum in a physiological medium.

MAJOR FINDINGS AND PROPOSED COURSE: The purification scheme developed over the past year has resulted in the removal of nearly all the extraneous contaminants with recoveries amounting to over 90%. The use of 90% ethanol in the formic acid solution enabled the solubilization of PSLI with the removal of the bulk of the mucins. Multiple repetitions of this extraction procedure were necessary due to lower yields with each step. The ion exchange resins eliminated a large amount of the remaining protienaceous material; PSLI failed to bind which indicates that the peptide is neutral and suggests that its termini are blocked. A large portion of the coloration was removed by passage through a preparative column of octyl silyl resin. The remaining yellow color was eliminated by chromatography on Bio-Gel P-4. Repeated recycling of the immunoreactive peak on ODS analytical HPLC columns using isocratic solvent systems will yield a pure peptide. The apparant height of the absorbance peak generally corresponds to the amount registered by RIA methods.

The proposed course of research concerns the final purification clean-up of the trial analyses and the application of the method to kilogram quantities of tissue which is a difficult task.. For example, the scaling up from 10 to 100 gm tissue requires about 5-times the amount of labor, time and solvents; for a kg lot, we speak of yet another one and a half orders of magnitude. Once microgram quantities of the peptide are homogeneous in purity, amino acid analysis will be done before sequencing, in order to facilitate the latter. From the known sequence, a synthetic peptide can be synthesized. This allows for mg amounts of the peptide for use in pharmacological and physiological experimentation, as an antigen in the production of new antisera (heterologous and monoclonal), in receptor binding analyses, and for infusion studies in vivo. The pharmacological studies will be in collaboration with D. V. Erspamer (Rome) since bioassays in vitro must be conducted with numerous tissues from several different species, in addition to in vitro studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The discovery and isolation of a new neuropeptide from mammalian tissue is indeed a rare find. But one that contains a degree of homology to an amphibian skin peptide is a unique situation in that its presumed biological function(s) may have been perpetuated throughout these millenia and may even predate anurans. Since PSLI is found associate with nerve fibers, Brunner's gland (a mucus secreting gland in the small intestine) and respiratory tissue, this suggests possible multiple roles for this peptide; in particular, it may lead to clues on cystic fibrosis or other maladies affecting salt and water balance. Furthermore, association with neuronal tissue paves the way for investigations on its perturbations by neurotoxicants.

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. 3: 329-341, 1982.

Lazarus, L. H., DiAugustine, R. D., Jahnke, G. D. and Hernandez, O.: Physalaemin: an amphibian tachykinin in human lung small-cell carcinoma. Science 219: 79-81, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50005-09 LBNT

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Noise and Ototoxic Agents on Energy Balance and Metabloism in Cochlea

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Teruzo Konishi Head, Medical Officer LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

LBNT

SECTION

Neurophysiology Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 to study ototoxic effects of furosemide and bromate on the cochlear potentials and ionic concentrations of the cochlear fluids.

Principal Investigator and All Other Personnel Engaged on the Project:

Teruzo Konishi	Head, Medical Officer	LBNT	NIEHS
Yukio Muratsuka	Visiting Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

Guinea pigs anesthetized with pentobarbital sodium were used. The endocochlear potential (EP) and K^+ activity in the perilymph and endolymph were measured in the basal turn of the cochlea with a pair of double barreled K^+ selective electrodes. The sound evoked responses were also recorded with differential electrodes placed in the basal turn. Furosemide was injected intravenously in the dose range of 25 to 100 mg/kg. Bromate was locally applied by perfusing the perilymphatic space with artificial perilymph containing 1mM of $NaBrO_3$. The rate of perfusion was 2μl min^{-1} and its duration was 60 min.

MAJOR FINDINGS:

(1) Effects of furosemide on cochlear potentials and K^+ concentrations in cochlear fluids.

The EP fell rapidly after injection of furosemide, reaching its minimum in less than 3 min. There was an initial rapid recovery followed by a gradual recovery. The maximum suppression of the EP was dose related and the negative EP was observed with 100 mg/kg of furosemide. The EP fully recovered during 90 min observation period. After progressive decrease in the cochlear microphonics (CM) the initial recovery of CM was more rapid than that of EP. But CM recovery pattern was then slower than the recovery of EP. The CM did not show full recovery 90 min after injection. The compound action potential was the most vulneable but in most cases the full recovery was observed 90 min after injection of furosemide.

The preliminary results show no substantial changes in K^+ concentrations in the endolymph and perilymph during 90 min observation period after injection of furosemide.

(2) Effects of bromate on cochlear potentials and K^+ concentrations of the cochlear fluids.

In control experiments in which the perilymphatic space was perfused with artificial perilymph, the EP showed a tendency to increase during perfusion. The mean increase was 10.3% at the end of perfusion. The normalized CM and AP relative to the preperfusion magnitude was 101.7% and 90.9% respectively at the end of perfusion. The CM and AP remained stable after perfusion. The endolymph K^+ activity was 123.8 + 8.3 Eq/l before perfusion and 127.4 + 9.0 mEq/l at the end of persusion. No substantial changes in the endolymph K^+ activity were observed after perfusion.

When the perilymphatic space was perfused with artificial perilymph containing 1mM NaBrO3, the EP showed an intial increase followed by a gradual decrease. The mean loss of the EP was 19% at the end of perfusion and 42% 60 min after end of perfusion. The CM amd AP showed a gradual decrease during perfusion and losses of CM and AP were 17.8% and 60% respectively. There was no recovery in CM and AP during 60 min after the end of perfusion.

We plan to study effects of furosemide and bromate on the cochlear potentials and ionic concentrations (K^+, Na^+ and Cl^-) of the cochlear fluids.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Although the ototoxicity of furosemide or bromate has been reported, the pathogenesis of these drug-induced cochlear dysfunction has not been extensively studied. The present study will make a significant contribution for the understanding of ototoxicity of furosemide or bromate.

PUBLICATIONS

Salt, A. N., Konishi, T. Functional Importance of Sodium and Potassium in the Gunia Pig Cochlea Studied With Amiloride and Titra Ethylammonium. Jap. J. Physical. 32: 219-230, 1982.

Konishi, T., Gupta, B. N. and Prazma, J. Ototoxicity of cis-dichlorodiammine platinum (II) in guinea pigs. Am. J. Otolaryngol. 4: 18-26, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50015-09 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Microwaves on Neural Response

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Donald I. McRee Research Physicist LBNT NIEHS

COOPERATING UNITS *(if any)*

Duke University, Durham, North Carolina

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Exposures of frog sciatic nerves to sine-wave modulated 2.45 GHz microwaves at frequencies of 16 and 32 Hz 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. Nerves treated with ouabain fatigued faster than non-treated nerves but no additional effect was found due to microwave exposure. These results would indicate that microwaves effects are due to interference in the operation of the Na-K pump since ouabain blocks the activity of the Na-K pump.

Principal Investigator and All Other Personnel Engaged on the Project:

Donald I. McRee	Research Physicist	LBNT	NIEHS
Howard Wachtel	Consultant	LBNT	U of Colorado

PROJECT DESCRIPTION

METHODS EMPLOYED:

Isolated neurons such as the sciatic nerve of the frog, and lobster ganglia 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 than 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.

In order to investigate the mechanism for the loss in vitality of frog sciatic nerves exposed to CW microwaves, nerves were treated with ouabain before being exposed. In this case no difference in loss of vitality was observed between the exposed and nonexposed nerves. Since ouabain blocks the activity of the Na-K pump, this result suggests that the microwave effect on nerve vitality are associated with the decay of ionic gradients that are normally maintained by active transport.

Additional experiments using frog sciatic nerves exposed to sine-wave modulated 2.45 GHz microwaves will be carried out to further study the concept that the effect is due to nonlinear behavior. Lobster nerves (a single nonmyelinated axon) will be used to study the effects of microwaves on membrane premeability to sodium and potassium ions. Selective ionic channel blockers will be utilized to study electromagnetic field interaction with membrane gating processes.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50017-10 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microwave Effects on Embryonic Development, Immunology and Fertility

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Donald I. McRee Research Physicist LBNT NIEHS

COOPERATING UNITS *(if any)*

Poultry Science Department, North Carolina State University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Non-Ionizing Radiation Workgroup

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Studies to determine the effects of microwave exposure as indicated above on brain development showed a slight development retardation in the cerebellar cortices. In fertility studies in adult Japanese quail exposed during embryogeny a decrease in spermatozoal numbers and motility were measured in exposed males and a decrease in fertility of approximately 10 percent occurred in eggs from mating of exposed male with either exposed or control females.

Principal Investigator and All Other Personnel Engaged on the Project:

Donald I. McRee	Research Physicist	LBNT	NIEHS
Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
James P. Thaxton	Consultant	Poultry Science,	NCSU
Carmen Parkhurst	Consultant	Poultry Science,	NCSU

PROJECT DESCRIPTION

METHODS EMPLOYED:

A. The first objective of this project is to determine the effects of 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. Effects of microwaves on brain development were studied. In the experiment irradiated and control embryos were removed from eggs on day 12,

13, or 14 of incubation and the cerebella were histologically examined. In order to examine the long-term effect of microwave radiation during embryogenesis on the cerebellum, some of the quail were allowed to hatch and were reared to eight weeks of age. Their cerebella were histologically examined, and the extent of dendritic arbores, the length of the stem of the primary dendrite, and the size of the perikaryon of Purkinje cells were measured in Gogli-Cox impregnated sections. In the irradiated embryos, a slight developmental retardation was found in the cerebellar cortices in terms of several morphological parameters. The effects included the growth and subsequent decline of the external granular layer, the 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 eight-week-old quail, no significant differences were noted between irradiated and control cerebella in the morphological measurements of Purkinje cells. In studies to determine the effects of embryonic exposure on fertility, the quail were allowed to mature and were mated until 23 weeks of age. At 23 weeks of age an assessment of the reproductive capacity of the males was performed. Spermatozoal numbers and motility in semen samples which were collected manually were reduced significantly ($P \leq 0.01$). However, spermatozoal viability and gross morphological characteristics in the exposed birds were not consistently different from the controls. Relative testicular weights were not altered significantly in the exposed males. Percentage of fertile eggs was significantly reduced when exposed males were mated to sham control females. The percentage of fertile eggs obtained from mating exposed males with sham control females was 72.5%, while the percentage of fertile eggs from mating of sham control males with sham control females was 80.4%. These data indicate that reproductive capacity in male Japanese quail is reduced when the birds are exposed to 2.45 GHz CW microwave radiation during embryogenesis.

B. Male Sprague-Dawley rats were exposed to 2.45 GHz microwave radiation at an incident power density of 10 mW/cm^2 daily for 3 hours from day 4 of pregnancy (in utero exposure) through day 40 postpartum, except for 2 days at the perinatal period. The animals were killed, and the brains 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 ventricles, the myelination of the corpus callosum, and the decline of the external germinal layer of the cerebellar cortex. In 40-day-old rats, quantitative measurements of neurons were also made. The spine density of the pyramidal cells in layer III of the somatosensory cortex, and the density of basal dendritic trees of the pyramidal cells in layer V were measured in Golgi-Cox impregnated specimens. In addition, the density of Purkinje cells

and the extent of the Purkinje cell layer in each lobule were measured in mid-sagittal sections of the cerebellum stained with thionin. There were no remarkable differences between microwave-exposed and control groups for any of the histological or quantitative parameters examined; however, the findings provide important information on quantitative measurements of the brain. The data from this study demonstrate that there is no significant effect on rat brain development due to microwave exposure (10 mW/cm^2) during the embryonic, fetal and postnatal periods.

Pregnant Sprague Dawley rats were exposed to 2.45 GHz CW radiation for 3 hr daily from day 4 thru 20 of pregnancy at an average power density of 10.3 mW/cm^2. At 2, 10, 20 and 30 days postpartum, several aspects of peripheral blood hematology of male and female pups were examined. No effects were noted on pregnancy rate, litter size or pup weight. However, the white blood cell numbers were lower in male and female pups and the differential cell counts altered in the female pups at 10 days postpartum. At 30 days of age, no differences were noted between the sham and exposed pups for any of the hematologic variables examined.

The research using the Japanese quail as a test system will be carried out under the research support contract at N.C. State University, (Contract No. NO1-ES-2-5019), "Research Support to Investigate Teratogenic Effects of Environmental Agents Using Avian Species". Future research on teratogenic effects of microwaves in rats will be directed to nervous system changes. A new project will be developed for that research. Therefore, this project will be completed in FY 1983.

PUBLICATIONS

Galvin, M.J., Thaxton, J.P., McRee, D.I. and Hall, C.A.: Immunity in late, juvenile and young adult Japanese quail as related to microwave radiation during embryogeny. Int J Radiat Biol 42: 673-677, 1982.

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.

McRee, D.I., Thaxton, J.P., and Parkhurst, C.R.: Reproduction in male Japanese quail exposed to microwave radiation during embryology. Radiation Research, (in Press).

Inouye, M. Galvin, M.J. and McRee, D.I.: Effects of 2450 MHz microwave radiation on the development of the rat brain. Teratology, (in Press).

Galvin, M.J., MacNichols, G. and McRee, D.I.: Effects of 2450 MHz microwave radiation during the gestational period on the postnatal hematology of rats. Cell Biophysics, (in Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50028-05 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Noise and Drugs on the Electrochemistry of the Cochlea

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)
Teruzo Konishi Head, Medical Officer LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurophysiology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Determination of electrochemical gradients across the cochlear hair cell membranes is essential for understanding the biophysical basis of cochlear transduction. The aim of the 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.

Principal Investigator and All Other Personnel Engaged on the Project:

Teruzo Konishi	Head, Medical Officer	LBNT	NIEHS
Yukio Muratsuka	Visiting Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

The measurement of the electrical potential and the K^+ activity difference across the cell membrane was carried out with double barreled ion selective liquid membrane electrodes. Exploration of the organ of Corti with ion-selective electrodes was carried out in guinea pigs anesthetized with pentobarbital sodium. After removal of the round window membrane an ion-selective electrode was advanced toward the organ of Corti by means of a piezo-electric microdriver. A 3M-KCl agar Ag/AgCl electrodes was placed in the intact neck muscles and used as a reference. During penetration of the organ of Corti with an ion-selective electrode, both electrical potential and ion-dependent potential were recorded on a strip chart recorder. The cochlear microphonics were also recorded from the potential barrel and photographed on a running film. A group of guinea pigs was exposed to broadband noise at 115 dBA for 7 to 10 days. A second group of guinea pigs was kept in a quiet environment. The criteria for a successful impalement of cell membranes were a) an abrupt appearance of negative dc potential and an associated increase in K^+ activity, b) maintenance of the plateau 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 penetration of hair cell membranes, in addition to the above criteria, was accompanied by a sudden increase of CM magnitude.

MAJOR FINDINGS:

(1) Electrochemical profile for K^+ across hair cell membranes.

Data were collected from 47 successful cell penetrations in normal and 38 in noise exposed guinea pigs. Out of these 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 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 electorchemical 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 guinea 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 electorchemical 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.

(2) Electrochemical profile for Na^+ and Cl^- across the hair cell membranes.

Our preliminary results showed that the Cl^- and Na^+ selective electrodes with very fine tip diameters (less than 1μm) do not accurately follow the Cl^- and Na^+ activity changes when sudden changes in the dc potential appeared at the moment of impalement of cell membranes. These artifacts appeared to be due to a long time constant of the ion selective electrode. We are trying to reduce the time constant of the ion selective electrodes so as to eliminate artifacts caused by an abrupt change of a background dc potential.

The experiments to determine the electrochemical profile K^+ across the hair cell membranes have been completed and a manuscript is in press. We plan to determine the electrochemical profile for Cl^- and Na^+ across the hair-cell membranes in normal and pathological ears in which the hair cells are degenerated by noise or ototoxic chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Determination of the electrochemical profile for ions 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 physiological mechanisms of noise induced cochlear damage.

PUBLICATIONS

Konishi, T. and Salt, A. N. Electrochemical profile for potassium ions across the cochlear hair cell membranes of normal and noise exposed guinea pigs. Hear. Res. In press.

Konishi, T. and Salt, A. N.: Electrochemical profile for potassium ions across the hair cell membranes. In Klineke, R. and Hartmann, R. (Ed.) Proceedings of Hearing - Physiological Bases and Psychophysics. Springer Verlag. In Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50033-05 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of 2450 MHz Microwave Radiation on the Cardiovascular System

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Michael J. Galvin Senior Staff Fellow LBNT NIEHS

COOPERATING UNITS *(if any)*
Physiology Department, Duke 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:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. However the response of atria to drugs was influenced by microwave exposure. Specifically the dose response curve for propranolol was shifted to the right during 10 and 100 mW/g exposure and the ability of propranolol to inhibit isoproteronol actions on rat atria was diminished by these exposure levels. The influence of microwave radiation on the response of isolated atria to other cardiotonic drugs are being undertaken. Also, certain biochemical and physiological parameters, which are indicative of cardiac integrity, 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 6 hr has no effect on mean arterial blood pressure or colonic temperature. However, there was a microwave induced bradycardia which was exhibited after 30 min of microwave exposure at 10 mW/cm^2 and persisted throughout the remainder of the 4 hr exposure period. None of the biochemical or hematologic indices examined were influenced by this exposure level.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
Donald I. McRee	Research Physicist	LBNT	NIEHS
Melvyn Lieberman	Consultant	LBNT	Duke University

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 tractile 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), hematologic and biochemical parameters were measured during 6 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 conraction 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. Preliminary data has shown the response of isolated atria to propranolol is affected. Specifically, the dose response curve to the drug is shifted to the right, (i.e., is less effective) during 10 mw/g microwave exposure. In addition, the ability of propranolol to inhibit the isoproteronal induced response was diminished during 10 and 100 mw/g exposure. These experiments will be extended to other drugs and other environmental agents.

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. Six hour exposure to 10 mW/cm^2 microwave radiation had no influence on MABP or temperature. However, there was a highly significant reduction in heart rate which

was evident within 30 min after the beginning of the exposure and persisted for the remainder of the experiment. In addition, a number of hematologic and biochemical 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 any of these parameters. This study will be completed in the coming year. The next study will examine the influence of microwave radiation on the biochemical and cardiovascular sequelae of endotoxin shock.

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 cardiovasular system can be evaluated more effectively. This research on the effects of microwaves on the cardivascular system is directed toward the mission of NIEHS to determine the health effects of physical factors in the environment.

PUBLICATION

Galvin, M.J., Dutton, M.S., and McRee, D.I.: Influence of 2.45 GHz microwave radiation on spontaneously beating rat atria. Bioelectromagnetics 3: 219-226, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50042-05 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparison of Impact Noise and Continuous Noise Effects on Cochlear Function

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Teruzo Konishi Head, Medical Officer LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

LBNT

SECTION

Neurophysiology Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Teruzo Konishi	Head, Medical Officer	LBNT	NIEHS
Reginald O. Cook	Acoustical Engineer	LBNT	NIEHS
Yukio Muratsuka	Visiting Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

Impact noise was generated by a mechanical impact noise generator. Continuous broadband 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 impact noise or broadband noise of equal energy. The suppression of tone induced responses (cochlear microphonics and compound action potential) during and following noise exposure was monitored.

MAJOR FINDINGS:

(1) The pneumatic impact noise generator as first designed and built was found to be limited in its output to about 100 dB peak SPL. The cause for the limit was deemed to be multiple air flow restrictions in the pnumatics. As a result a new high capacity system was implemented using larger pistons and air line. The durability of this modified impact noise generator will be tested and the acoustic properties will be measured.

(2) The control experiments have been initiated. The stability of the cochlear microphonics (CM) and compound action potential (AP) were tested in anesthetized guinea pigs in quiet environment. The input-output function of CM in response to 500Hz tone bursts recorded in the cochlear basal turn showed a gradual decrease in amplitude. The mean loss of CM sensitivity expressed by sound intensity required to elicit 100μV peak to peak CM was 4.5 dB during 90 min observation period. The mean loss of CM sensitivity in response to 500Hz tone bursts recorded in the third turn of the cochlea was 2.4 dB. The variation of AP was greater than CM. The input-output function of AP in response to 5kHz bursts showed a slight loss of the sensitivity in low intensity range of stimuli but remained unchanged in high intensity range during 90 min observation period. These data will be used as baseline data to which CM suppression produced by noise exposure can be compared.

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 to 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50058-04 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microwave Effects on Fetal Development in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Donald I. McRee Research Physicist LBNT NIEHS

COOPERATING UNITS *(if any)*
Research Triangle Institute

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Non-Ionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Peripheral blood and bone marrow samples were obtained on day 18 of pregnancy from the dams. Total leukocyte and differential leukocyte counts of peripheral blood samples were not affected. No effects were noted in either the erythroid or myeloid mitotic index of bone marrow samples. Studies specifically designed to determine the effects on development preimplantation embryos were carried out at power densities of 9 mW/cm^2 and 19 mW/cm^2 on either day 2 or 3 of pregnancy. Heat stress due to exposure caused stunted development of embryos, but no remarkable effects were found on the development of preimplantation embryos.

Principal Investigator and All Other Personnel Engaged on the Project:

Donald I. McRee	Research Physicist	LBNT	NIEHS
Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
Peter Nawrot	Research Scientist	Health & Welfare Canada	
Minuro Inouye	Research Scientist	Institute of Developmental Research, Kasugai, Japan	

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 significant decrease in implantation sites per litter and average fetal weight. In our previous 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. Peripherial blood and bone marrow samples were obtained on day 18 of pregnancy from the dams. Total leukocyte and differential leukocyte counts of peripheral blood samples were not affected. No effects were noted in either the erythroid or myeloid mitotic index. Therefore exposure of pregnant mice to microwave radiation under the conditions of this experiment had no effects on the measure indices of hematopoiesis.

The development of preimplantation embryos after exposure to microwave radiation was studied. Female CD-1 mice were induced to superovulate, mated, and exposed to 2.45 - GHz microwave or sham radiation for 3 h at power densities of 9 mW/cm^2 and 19 mW/cm^2 on either day 2 or 3 of pregnancy (plug day was considered day 1). Another group of mice was exposed to heat stress by placing the dams in an environment room at an ambient temperature of 38°C and relative humidity at 62% for 3 h on day 2 of pregnancy. All groups were euthanized on day 4 of pregnancy and embryos were recovered by flushing excised uterine horns. Embryos were examined for abnormalities and classified by the development stages. They were then treated with hypotonic solution and dissociated for counting blastomeres. Heat stress caused stunted development of embryos, but no

remarkable effect of microwave radiation could be found on the development of preimplantation embryos. 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

Inouye, M., Matsumoto, N. Galvin, M.J., and McRee, D.I.: Lack of effect of 2.45 - GHz microwave radiation on the development of preimplantation embryos of mice. Bioelectromagnetics 3: 275-283, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50060-04 LBNT

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microwave Interactions with Cells and Cellular Components

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael J. Galvin Senior Staff Fellow LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and sub-cellular level, to observe any effects of this interaction, and to relate the amount of microwave energy absorbed to the effect. The biological specimens have included mitochondria, cardiac cells, lysosomes, and cellular enzymes. For these experiments, a waveguide apparatus has been used which can maintain the specimens at 30°C at specific absorption rates up to 100 mW/g. For tightly or loosely coupled mitochondria using succinate as substrate, no effect of exposure was observed on respiration states 1-4 or the respiratory control index. When glutamate was used as substrate, no effects were observed at 10 mW/g. However, in the loosely coupled mitochondria, the 100 mW/g exposure produced an increase in respiration states 2 and 4 and a decrease in the respiratory control index. The results suggest the function of loosely coupled mitochondria can be affected at high power levels of microwave radiation.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
Donald I. McRee	Research Physicist	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

Cells and cellular components were exposed to 2450 MHz microwave radiation using a waveguide exposure apparatus developed in this laboratory. For mitochondria, exposure levels were 10 and 100 mW/g. In addition, mitochondria obtained by two different isolation methods were used. One method yielded mitochondria with respiratory control indexes >5 and were considered tightly coupled. The other yielded mitochondria with respiratory indexes <4 and were considered loosely coupled. Mitochondria were exposed for 5 minutes prior to and throughout stimulated respiration. Respiration was stimulated by one of two substrates: succinate or glutamate.

MAJOR FINDINGS AND PROPOSED COURSE:

For tightly coupled mitochondria, there were no differences in respiration rates for either substrate or power level. The loosely coupled mitochondria also exhibited no changes at either power level when succinate was used as substrate. However, when glutamate was used respiration rates 2 and 4 increased at 100 mW/g and the respiratory control index decreased. These changes were not evident at the 10 mW/g exposure. These experiments will be expanded to include the effects of drugs and toxic chemicals on mitochondrial function during microwave exposure.

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

Dutton, M.S., Galvin, M.J., and McRee, D.I.: In vitro effects of microwave radiation on rat liver mitochondria. Bioelectromagnetics, submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50076-02 LBNT

PERIOD COVERED
October 1, 1981 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Noise and Drugs on Water Control of the Cochlear Fluids.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Teruzo Konishi Head, Medical Officer LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
LBNT

SECTION
Neurophysiology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Teruzo Konishi	Head, Medical Officer	LBNT	NIEHS
Philip E. Hamrick	Radiation Safety Officer	SFTY	NIEHS
Hiromi Ueda	Guest Worker	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

Guinea pigs were anesthetized with pentobarbital sodium. The periliymphatic 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 mCi/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, 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.

The concentrations of Na, K and Cl and osmolarity of the cochlear fluids, blood and cerebrospinal fluid were measured in non-perfused and perfused cochlea. To increase osmolarity of blood 3g/kg of glycerin (glycerol) was injected intravenously as a 40% aqueous solution. Samples of blood were taken before and after injection of glycerin. The osmolarity of the cochlear fluids 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:

(1) Osmolarity of the cochlear fluids, the preliminary results showed that the osmolarity of the endolymph was 301.4+ 5.9mOsM which was slightly higher than the osmolarity of the perilymph (287.6I5.3 mOsM). The osmolarities of blood serum and cerebsospinal fluid were 281.8 $\pm$ 11.8 mOsM and 294.5 $\pm$ 8.2 mOsM respectively. The osmolarity of the blood serum increased rapidly after injection of glycerin and reached the maximum between 15 and 30 min. The osmolarity of the blood serum decreased gradually and remained high 2 hrs after the injection.

(2) Diffusional permeability to water of the endolymph-perilymph barrier. When the perfusate was introduced into the scale tympani of the basal turn of the cochlea, 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 1 min. The concentration of 3H in fluid samples from the scala vestibuli had a tendency to increase exponentially, the time constant being (0.45 $\pm$ 0.06) min. The concentration of 3H in the endolymph increased

rapidly and reached 70% 5 min after perfusion commenced and thereafter the rate of increase was slow. The transport rate constant for water was (0.90 ± 0.38) min^{-1} which was 100 times greater than that for k^+.

We plan 1) to study effects of glycerin administration on the osmolarity of the cochlear fluids, 2) to determine the diffusional permeability coefficicent for water of the endolymph-perilymph barrier when the osmolarity of the perilymph is altered, and 3) to study possible alterations of water control in the cochlear fluids in noise or drug treated guinea pigs.

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 chemical or physical agents.

PUBLICATIONS

Konishi, T. Ion and Waster Control in Cochlear Endolymph. Am. J. Otolaryngol. 3: 434-443, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90011-05 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobehavioral Toxicity of Organometals and Related Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

H. A. Tilson Head, Neurobehavioral Workgroup 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:
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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
C. F. Mactutus	Staff Fellow	LBNT	NIEHS
C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
J. S. Hong	Pharmacologist	LBNT	NIEHS
T. J. Walsh	Staff Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Characterization of Organometal Neurotoxicity

Our initial studies involve a comparison of the neurobehavioral effects of four alkylmetals (triethyl and trimethyl tin and lead). Effects on several aspects of neurobehavioral functioning (i.e., motor, reactivity, associative, nonassociative) are being determined. These experiments are intended to examine the nature and persistence of the behavioral effects produced by representative organometals.

a. Preliminary Neurobehavioral Assessment

A battery of tests is being used to directly compare the effects of trimethyl and trimethyl tin and lead. The results of these experiments will indicate the similarities and differences between the selected alkylmetals in terms of the presence or absence of an effect, as well as time course and dose responsiveness of the observed effects. This information will be used in establishing dosing parameters in subsequent experiments.

b. Spatial Learning in the Radial-Arm Maze

The radial-arm maze (RAM) procedure is a complex spatial learning task in which animals must "remember" a list of previously entered and non-entered feeders during a free choice test session. Several converging lines of evidence indicate that the septo-hippocampal-entorhinal circuit is a necessary substrate for performance of this task; thus, if an organometal such as TEL alters limbic forebrain function, it should interfere with performance in the RAM.

c. Avoidance Learning

One of the most consistent behavioral observations following limbic system lesions is task-specific alterations in avoidance learning. Lesions of both the septal and hippocampal formations have repeatedly been shown to impair passive and facilitate two-way active avoidance responses. Thus, if alkylmetals such as TEL affect limbic system function, they should have similar effects on avoidance performance. Changes in avoidance performance

should also occur in the absence of alterations in responsiveness to electric footshock as measured by standard flinch-jump procedures.

2. Limbic System as a Site of Toxicity

a. Lesioning Studies

The purpose of the lesioning studies is to provide a comparison of the behavioral effects of systemically administered alkylmetals, such as TEL, with those of cytotoxicants whose mechanism of action is known and with those of lesions produced by electrochemical means. Information derived from these experiments will be useful in determining the selectivity of any neuropathological effects produced by the organometals.

b. Developmental Studies

The major purpose of these experiments is to characterize the neurotoxic effects of organometals in the developing animal. Initial studies establish the sensitivity of neonatal rats to the acute effects of alkylmetals, while subsequent studies will determine dose- and time-related effects on neurobehavioral function.

3. Assessment of Effects on Neurotransmitter Systems

One aspect of limbic function possibly important for understanding the neurobiological basis of organometal toxicity is the role of acetylcholine in the regulation of behavioral processes. Most limbic nuclei and all regions of the cerebral cortex are heavily cholinergically innervated and administration of anticholinergic drugs produces behavioral deficits similar to those observed following administration of some organometals. We are currently studying the effects of AF64A, a ethyl choline mustard aziridinium ion cytotoxic for cholinergic nerve terminals, on behavioral parameters known to be affected by exposure to organometals (i.e., performance in the RAM and antinociception).

4. Neurological Basis of Selected Effects

The purpose of these studies is to study in detail the possible neurobiological substrate responsible for selected neurotoxic effects These are essentially mechanism of action studies and use a variety of methods and strategies. At the present time, we are investigating the possible causes for alkylmetal-induced antinociception. We are investigating the hypothesis that organometals such as triethyl lead increase latencies to respond to thermal noxious stimuli by disrupting limbic processes leading to an altered behavioral reactivity to environmental stimuli.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Comparison of Representative Organometals

Preliminary experiments have been completed using a battery of neurobehavioral tests to determine the effects of TEL. In these studies, we found that TEL produced a phase of hyperexcitability followed by a phase of hypoexcitability. Generally, TEL altered responsiveness of rats to noxious and nonnoxious stimuli; operant titration studies and flinch-jump measurements indicated that TEL did not affect response thresholds. TEL-exposed rats were found to perform better in a two-way shuttle box procedure and pilot work suggested that TEL impaired retention of a step-through passive avoidance task. Additional studies have indicated that repeated exposure to triethyl lead or tetraethyl tin produces suppression of neuromotor function and responsiveness to noxious and nonnoxious stimuli. These data have provided the basis for selection of tests and time points for measurement in a large study directly comparing the effects of other organometals.

In addition, other experiments have indicated that TEL affects several indices of neurochemical function in limbic forebrain sites and that these effects were associated in time with concurrently measured behavioral effects. Studies are now underway to compare the effects of other alkylmetals with those of TEL.

2. Limbic System as a Site of Toxicity

The main accomplishment in this area has been a series of studies in which TEL has been administered neonatally and behavior assessed at various ages. Injection of TEL on day 5 of age has been shown to result in alterations in startle reactivity and motor activity, as well as other changes in responsiveness. Preliminary data suggest that TEL results in degeneration of CA3 cells in the hippocampus, as well as producing cell death in other limbic forebrain areas at higher doses. The neurobehavioral effects of neonatal exposure to TEL differ from those following exposure on day 5 postnatally to TET.

3. Assessment of Neurotransmitter Systems

Studies conducted thus far with AF64A to selectively lesion cholinergic neurons in the hippocampus have shown that the behavioral effects of AF64A resemble in many cases those produced by systemic administration of TEL and TMT. Generally, intraventricular administration of AF64A impairs passive avoidance retention and results in alterations in behavioral reactivity. Neurochemical studies are now underway to verify the selectivity of the cholinergic lesion relative to that produced by TEL.

4. Neurological Basis of Selected Effects

Initial studies indicated that TEL, like TMT, increases latencies to respond on the hot plate. Subsequent experiments have found that TMT and TML produce similar effects, although the time-course of the effects appear to differ. Moreover, it has been observed that the antinociception produced by TEL can be differentiated in terms of topography from that of morphine, suggesting that the effect is not opiate-like. Support for this hypothesis comes from additional studies indicating that TEL-induced changes in hot plate latencies can be attenuated by chloriazepoxide, but not by optimal doses of naloxone, BC-105, or trihexyphenidyl. Finally, TEL-induced antinociception appears to be modifiable by environmental manipulations such as the entent of post-treatment handling and/or repeated exposure to the initially novel hot plate.

5. Characterization of Organometal Neurotoxicity

At the present time, the effects of TEL on RAM performance and avoidance responding are under investigation. Future studies will concern the effects of the other alkylmetals targeted for intensive study (i.e., trimethyl lead, trimethyl tin, triethyl tin).

Several experiments are planned to investigate the limbic system as a site of toxicity for alkylmetals. For example, ibotenic acid is a cytotoxicant known to attack cell bodies selectively; this agent will be administered into targeted sites and the resulting behavioral effects compared to those of TEL and other alkylmetals. Likewise, electrolytic lesions will be stereotaxically placed into limbic sites in an attempt to mimic the lesion produced by the systemic administration of TEL and other alkylmetals. In most cases the site of where the cytotoxicant is injected or the electrolytic lesion is made will depend on results from neurochemical and neuropathological studies suggesting some degree of selectivity for a given site. Finally, if some correspondence between the behavioral effects of systemically administered alkylmetals and experimentally induced lesions is attained, additional studies in which the alkylmetals are injected directly into the targeted site will be performed.

We also plan to extend our observations using the developing organism as the target population. In addition to the initial characterization of neonatal toxicity of TEL, subsequent studies will determine dose- and time-related effects on neurobehavioral function, the distribution of TEL within the CNS, and the neuropathological consequences with particular respect to the limbic system. Moreover, the hypothesis of alterations in trace metals as underlying the regional vulnerability of nervous tissue will be investigated. The generality of findings made with TEL will be determined for other alkylmetals, especially trimethyl lead.

Current experiments are investigating the effects of the organometals on cholinergic systems. Future studies will be concerned with effects on other systems found to be altered by the organometals. Based on preliminary data from the Neuropharmacology and Neurochemistry Workgroups, it is likely that effects on neuropeptides (i.e., met-enkephalin) and benzodiazepines/GABA will be studied in greater detail.

Currently, we are investigating the neurological basis for the antinociception produced by the organometals. Future studies will be concerned with the effects of the organometals on other neurological functions, such as changes in responsiveness to nonnoxious stimuli. Of course, effects on learning/memory processes will be extended in an attempt to determine the precise nature of any observed associative deficits (i.e., short-term vs long-term memory).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Organometals have many industrial and occupational uses. Understanding their mechanism of action will lead to scientifically based strategies for treatment.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90019-04 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Neurotoxicants on Neuropeptides and Neurotransmitter of Rat Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. S. Hong Head, Neuropharmacology Workgroup LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neuropharmacology Workgroup

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to characterize the effect of chlordecone or estrogen on the rat pituitary level of (Met^5)-enkephalin (ME) and to examine the possibility that the modulation of pituitary ME by chlordecone may be mediated through its estrogenic-like activity. The anterior pituitary enkephalin system is regulated by circulating sex hormones. Androgen increases, whereas estrogen decreases, the level of pituitary enkephalin. Chlordecone exerts robust effects on the hypothalamo-pituitary axis such as pituitary enkephalin system and the estrogenic activity of this insecticide may mediate some of its effects on the neuroendocrine function. Both estrogen and chlordecone decrease the level of enkephalin in the pituitary but increase the level of this peptide in the striatum. Since estrogen exerts antidopaminergic activity both in the striatum and in the pituitary plus the fact that the enkephalin systems in these two regions are regulated by dopamine, it is possible that the effect of estrogen and chlordecone on the pituitary and striatal enkephalin systems is mediated through the dopaminergic system.

Principal Investigator and All Other Personnel Engaged on the Project;

J. S. Hong	Head, Neuropharmacology Workgroup	LBNT	NIEHS
K. Yoshikawa	Visiting Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

The effect of chlordecone or estrogen on the pituitary ME levels was examined in adult Fischer rats. Animals received a single injection of chlordecone (75 mg/kg, ip) or estrogen (0.5 mg/kg, im) and were killed at various time intervals post-dose. Homogenates of the anterior pituitary and the striatum were fractionated by HPLC and levels of ME were determined by RIA.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Regulation of Pituitary ME Levels by Sex Hormones

   In order to demonstrate the estrogenic activity of chlordecone on pituitary ME the effects of estrogen (and androgen) on this peptide level were determined. The main findings from this study are briefly summarized:

   a. A distinct sex-related difference of ME was found in the anterior lobe but not in the neurointermediate lobe of the rat pituitary. Levels of ME in the anterior pituitary of male rats was four-fold as much as that of female rats.

   b. Estradiol administration caused a marked decrease of ME only in the anterior lobe of male rats. Castration of male rats decreased the pituitary level of ME only in the anterior lobe and the diminished level of ME was partially restored by the administration of dihydrotestosterone. On the other hand, ovariectomy caused a significant increase of ME level in the anterior lobe of female rats, and this increase was completely prevented by the administration of estradiol.

   c. A series of experiments were conducted to examine the possibility that the sex-related difference in pituitary ME levels may be related to imprinting during the sexual differentiation of the brain. The results suggested that the pituitary ME levels were not affected by the imprinting process. In contrast, the sex-related difference in the levels of pituitary substance P was determined during the period of sexual differentiation of the brain.

2. Comparison of the Effect of Chlordecone and Estrogen on the Pituitary Levels of ME

A time course study following a single injection of chlordecone or estrogen showed that these two compounds exert almost identical effects on the pituitary levels of ME. Furthermore, like estrogen, the effect of chlordecone on the pituitary level of ME was regionally specific (only the anterior lobe but not the neurointermediate lobe) and sex specific (only males but not females).

These results strongly suggest that chlordecone modulates the pituitary level of ME via its estrogenic activity. Thus, in addition to reproductive systems, the HPA appears to be another primary target sensitive to the estrogenic activity of chlordecone.

3. Comparison of the Effects of Chlordecone and a Synthetic Estrogen on the Plasma Levels of Luteinizing Hormone (LH) and Prolactin (PRL)

To investigate the possibility whether chlordecone may mimic the effects of estrogen on other pituitary hormones, we determined the plasma levels of LH and PRL in rats treated with chlordecone or a synthetic estrogen, diethylstibestrol (DES). Replacement of DES in ovariectomized rats produced anticipated results, i.e., an increase in PRL level and a decrease in LH level. Chlordecone mimicked the effect of DES on these two hormone levels although the potency was less than that of DES. These results extend the similarity between estrogen and chlordecone in the modulation of pituitary hormones and further support the notion that chlordecone exerts potent estrogenic activity on the HPA.

4. Possible Involvement of the Dopaminergic System in Mediating the Effects of Estrogen or Chlordecone on Pituitary ME levels

It is well documented that estrogen treatment increases plasma PRL level via its antidopaminergic trait. Since chlordecone and estrogen treatment increased plasma PRL levels and decrease pituitary levels of ME, it is possible that dopaminergic neurons mediate these effects. To investigate this possibility, haloperidol, a dopamine receptor blocker, was administered to adult male rats. Repeated injections of haloperidol reduced the pituitary level of ME in a manner similar to those after estrogen or chlordecone treatment. This result is consistent with the hypothesis that dopaminergic neurons mediate the estrogen or chlordecone-induced decrease in the pituitary level of ME. This same phenomenon has also been observed in the brain. We have previously reported that repeated injections of haloperidol increase the striatal level of ME and speculated that antidopaminergic effect may be related to this effect. Further studies indicated that, like haloperidol, both chlordecone and estrogen increase the striatal level of ME. It is interesting to note that, despite differences in structure and action, haloperidol, estrogen, and chlordecone produce similar effects on the pituitary and striatal levels of ME, although the changes of this peptide in these two regions are opposite. Since the antidopaminergic activity may be the only common denominator of these compounds, our findings

suggest a possible involvement of the DA system in the action of these compounds on brain and pituitary ME.

The proposed research is an attempt to study in greater detail the modulation of brain and pituitary ME systems by chlordecone, estrogen and haloperidol. Future work will focus on the following three aspects.

1. Site of action

It is not known whether the modulation of brain and pituitary ME levels by these three compounds represent a direct effect on ME or may be mediated by other neurotransmitters or hormones. Studies involving an in vitro pituitary cell culture or direct microinjection of these three compounds into various brain regions will be conducted to answer this question.

2. Mode of action

Changes in the levels of ME in the pituitary and in the striatum after chlordecone, estrogen and haloperidol may be due to a change in the biosynthesis or in the release, since either process may alter the level of this peptide. Using an in vitro cell free translation technique, we will develop a method for the measurement of biosynthesis of ME. This method will permit us to examine the dynamic change of ME after treatment with these compounds.

3. Functional implications

Experiments will be designed to explore some functional implications of alterations in the levels of ME elicited by chlordecone, estrogen and haloperidol. The purpose of this approach is to explore possible roles of ME in mediating the actions of these three compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Illucidation of the estrogenic activity of chlordecone in affecting the pituitary enkephalin system may help us to learn more about the actions of this insecticide on the hypothalamo-pituitary function. This will lead to a better understanding of the toxic effects of chlordecone on neuroendocrine functions, such as reproductive failure which is one of the major symptons observed in patients exposed to this insecticide.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90024-04 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neurobehavioral Toxicity of Chlordecone

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
H. A. Tilson Head, Neurobehavioral Workgroup 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:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

H. A. Tilson	Head, Neurobehavioral Workgroup	LBNT	NIEHS
C. F. Mactutus	Senior Staff Fellow	LBNT	NIEHS
T. A. Burne	Psychologist	LBNT	NIEHS
J. S. Hong	Pharmacologist	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Characterization of Neurobehavioral Toxicity in an Animal Model

a. Tremor

A spectral analysis procedure to measure tremor in a conscious, freely moving rat was developed. Briefly, this technique involves placing a rat atop a Plexiglas platform which is attached to a force transducer. The output from the transducer is transmitted to a commercially available (Hewlett-Packard) spectral analyser. The waveform generated by tremoring rats in averaged over a 2.5 min period and a fast fourier analysis is performed providing a measure of power (-dBV) of movement as a function of wavelength. Measures of tremor are taken as a function of dose and time following injection. Once initial pharmacological parameters are established, then additional studies are performed to study the neurobiological substrate responsible for the tremor. Such studies consist of measuring brain neurotransmitters or neuromodulators in tremoring rats and using pharmacological and/or physiological intervention to antagonize or exacerbate the tremor.

b. Hyperexcitability

Startle responsiveness to an acoustic stimulus is quantified by placing the rats atop a platform attached to a force transducer located in a sound-attenuated environment. An 8KHz tone is presented at 110 dB(A) against a 75 dB white noise background for a given number of trials (usually 20); the stimulus duration is 200 msec and the inter-stimulus interval is quantified by a commercially available unit (Columbus Instruments); typically the amplitude of the first response and the average response per trial are recorded. Studies concerned with the phenomena of habituation and sensitization employ slightly different protocols and data analysis. Once time- and dose-response functions for hyperexcitability are established, additional studies are performed to determine the neurobiological substrate responsible for the effect.

c. Associative Effects

The purpose of these studies is to determine the effects of chlordecone on the associative processes of learning and memory. Both aversively and appetitively motivated tasks are employed. If those

studies indicate an impairment in learning and/or retention following acute and repeated administration of chlordecone, then additional experiments are performed with the addition of appropriate control groups to determine whether these effects are dissociable from changes of a nonassociative nature, e.g., activity. Providing alterations of an associative nature are confirmed, time- and dose-response functions are determined.

1. Active Avoidance

The effects of pharmacological and toxicological agents on acquisition are frequently investigated in a task in which the animal has to make a response in order to be reinforced. The two-way shuttle box procedure involves a discrete trial contingency in which an animal must shuttle frcm one compartment to the other in order to avoid or escape electric footshock applied to the grids of the chamber. One-way avoidance procedures involve a similar contingency, but the direction of the response is limited, thereby eliminating the conflict of having to return to the area in which shock has just occurred.

2. Passive Avoidance

A multiple measure passive avoidance task is employed to assess the ability of the chlordecone-exposed rat to learn to withold a response to avoid being shocked. If effects of chlordecone on acquisition or retention (24 or 144 hrs) are observed, additional measurements of nonassociataive factors (i.e., changes in sensitivity to electric footshock) are made.

3. Radial Arm Maze

Short-term memory deficits are assessed using food reinforcement in the RAM, a complex spatial learning task in which the animals must maintain in "working memory" the series of previously entered and nonentered feeders to obtain all the food pellets.

2. Developing Animal as a Model for Chlordecone Neurotoxicity

It is generally believed that developing animals are sensitive to the effects of hormonally active agents, especially if exposure occurs during the phase of neuroendocrine differentiation. The purpose of these experiments is to determine the neurotoxic effects of chlordecone in the developing rat and to attempt to establish if these effects are related to alterations in the HPA during the postnatal phase of neuroendocrine differentiation.

Neonates are injected with various doses of chldordecone and the short and long-term effects are investigated. These studies are intended to determine if neonates are affected by chlordecone in a manner similar to

that of adults, i.e., effects on tremor, startle. Pituitary-adrenal responsiveness is assessed by measuring serum corticosterone at various times following exposure to stressful test conditions.

MAJOR FINDINGS AND PROPOSED COURSE

1. Animal Model

a. Tremor

The dose- and time-response characteristics of chlordecone-induced tremor were characterized in adult rats. Chlordecone produces measureable tremor approximately 1 hr after the administration of 50-100 mg/kg. The peak time of effect was found to be 5-12 hrs postdosing and the duration was measured in terms of days (i.e., rats receiving up to 100 mg/kg of chlordecone displayed prominent tremor for up to 1 week postdosing, after which they were sacrificed). These observations correspond to the relatively long half-life observed by other laboratories (i.e., approximately three weeks for the first phase).

The role of neurochemical transmitter systems in the expression of chlordecone-induced tremor was examined. The serotonergic and cholinergic systems appear to be involved in chlordecone-induced tremor, as they are for DDT. Additional studies have indicated that catecholaminergic-containing systems are not necessary for the expression of chlordecone-induced tremor. Moreover, it was thought that tremor might be cerebellar in origin, but lesioning the climbing fibers into the cerebellum with 3-acetylpyridine did not attenuate and appears to have exacerbated chlordecone-induced tremor. Subsequent pharmacological studies have indicated that the brain stem and spinal cord are necessary for the expression of chlordecone-induced tremor.

b. Hyperexcitability

Preliminary experiments in our laboratory indicated that acute or chronic administration of chlordecone results in an exaggerated startle response to an air puff and acoustic stimulus presented on a single trial. Increased responsiveness produced by chlordecone does not appear to generalize to all stimuli, since we also found that responsiveness to noxious stimuli (thermal, electric) is not altered in chlordecone-exposed rats.

c. Associative Processes

Preliminary work completed in our laboratary has indicated that chlordecone-exposed rats acquire a discrete trial one-way shock avoidance task at the same rate as controls. However, when the animals were retested, there was some evidence of impaired retention. Subsequent experiments indicate that rats chronically exposed to chlordecone acquire and retain a two-way shuttle box response as well as controls,

suggesting that chlordecone-exposed animals may have a retention deficit detectable only under certain testing conditions.

2. Developing Animal as a Model of Chlordecone Neurotixicity

Preliminary experiments have dosed pups with 0.2 to 1 mg of chlordecone on day 4 postnatally. It was found that exposure to chlordecone on day 4 did not affect neurological function as assessed by a battery of tests on 30 and 100 days of age, but did alter baseline responding on a two-choice, visual discrimination task. Recently, we found that neonatally exposed rats displayed a memory deficit for a passive avoidance response over a 144 hr retention interval and that serum corticosterone levels were elevated in chlordecone-exposed animals immediately after the last retention test of each experiment. In subsequent experiments, it was observed that the resting levels of corticosterone were decreased significantly in prepubsecent and adult animals exposed to chlordecone via the mother during gestation and lactation or following a single injection of chlordecone on day 4 postnatally. These data led to the hypothesis that developmental exposure may interfere with neuroendocrine differentiation and alter the development of the hypothalamic-pituitary-adrenal axis, which may have long-term effects on behavior.

FUTURE PLANS AND RESEARCH DEVELOPMENT

1. Characterization of Neurotoxicity in an Animal Model

a. Tremor

The proposed experiments will attempt to elucidate the relative contribution of major aminergic, tryptaminergic, and cholinergic pathways to chlordecone-induced tremor. Initial experiments will involve general depletion of brain serotonin, norepinephrine or dopamine and acetylchloine by pretreating animals with intracerebral infusions of specific cytotoxicants (5,7-dihydroxytryptamine, 6-hydroxydopamine and AF64-A). After allowing time for degeneration of targeted pathways, animals will be challenged with chlordecone. The results of these experiments will be the basis for subsequent experiments in which specific portions of biogenic amine pathways are lesioned with cytotoxicants or electrolytic lesions and the effects on chlordecone-induced tremor assessed.

Addaditional studies are planned to isolate the possible site of chlordecone-induced tremor. These experiments would involve microinjecting chlordecone into various regions of the brain alone and in combination with pharmacological agonists and antagonists.

b. Hyperexcitability

Previous work in this laboratory has utilized a single exposure to a novel stimulus, so assessment of chlordecone on startle habituation will be made. Another series of experiments will determine effects of chlordecone on sensitization. Taken together, these studies may help determine whether chlordecone is affecting the sensory or the motor side of the startle reflex circuit.

Additional experiments with chlordecone-induced startle will involve the study of various neurotransmitter systems. Various pharmacological agents will be used to antagonize or exacerbate chlordecone-induced alterations in startle.

c. Associative Effects

Experiments to determine the effects of chlordecone on passive avoidance and RAM acquisition will be initiated.

2. Chlordecone Neurotoxicity in the Developing Rat

Currently, experiments are in progress to determine the effects of neonatal exposure on tremor, startle responsiveness, and learning/memory processes. Additional studies will be performed to determine the generality of these findings to other times of injection (i.e. day 11 postnatally) and other vehicles for administering chlordecone (i.e., corn oil). Because of the observation that chlordecone alters the retention of a passive avoidance task when tested about the time of weaning, the effects of chlordecone on acqusition and retention in another learning paradigm (i.e., two-way shuttle box) will be determined.

Early work by this laboratory has indicated that chlordecone-induced behavioral and neuroendocrine alterations may be due to activational effects (i.e., the direct result of the presence of chlordecone in the tissue). The potential for long-term or organization effects is also suggested by behavioral, physiological and neuroendocrine effects. A systematic examination of the long-term effects of neonatal exposure will be performed using the same tasks as employed in the preweaning period. The influence of early experience is believed to have a marked influence on subsequent behavioral development and it should influence the expression of the long-term toxicity resulting from neonatal exposure to chlordecone. This possibility will be explored systematically.

Our laboratory has found that neonatal exposure to chlordecone can alter the capability of the animal to adapt to changing environmental (i.e., reinforcement contingencies) factors. Thus, learning situations other than the passive avoidance procedure will be used to study the effects of chlordecone on this neurobehavioral process (i.e., short-term memory in the radial arm maze).

Finally, the use of pharmacological challenges will be considered where appropriate to corroborate the existence of an observed neurochemical alteration by the Neuropharmacology Workgroup or to assess possible alterations in pharmacological responsiveness as suggested by behavioral data. For example, altered turnover of serotonin in developmentally exposed animals suggests that they might exhibit altered responsiveness to serotonergic agonists or antagonists.

Preliminary data and our initial reports have indicated that neonatal exposure to chlordecone alters the behavioral reactivity of animals when

Preliminary data and our initial reports have indicated that neonatal exposure to chlordecone alters the behavioral reactivity of animals when tested during the preweaning and immediate post-weaning phase. The hypothesis that chlordecone acts directly or indirectly on the adrenal gland and the feedback regulation of the hypothalamic-pituitary-adrenal axis (HPA) to interfere with neuroendocrine differentiation of the developing animal will be explored. Initial studies will determine the distribution and half-life of chlordecone in the neonate and the time-course of serum corticosterone alterations following injection of chlordecone. β-Endorphin levels will also be measured as a possible index of ACTH release. Additional studies will attempt to mimic the behavioral effects of neonatal exposure to chlordecone by administering agents with known pharmacological and/or hormonal effects. Exogenous steriods (corticosterone or 17-β-estradiol) will be studied as will be o,p'-DDT and p,p'-DDT. These latter two agents are of interest because like chlordecone they are organochlorine insecticides with potentially the same or similar mechanisms of action. Perhaps moré important is the fact that both isomers are neurotoxic (i.e., produce tremors and hyperexicitability, but o,p'-DDT has only 30% the estrogenicity of p,p'-DDT. To the extent that hormonal alterations induced by neonatal chlordecone underlie the neurobehavioral and/or functional toxicity of the HPA axis, it is expected that the effects of o,p'-DDT, but not p,p'-DDT, will more closely resemble those of chlordecone.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90030-03 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Toxicants on Membrane-Related Neurochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Stephen C. Bondy Head, Neurochemistry Workgroup LBNT NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The current emphasis of this project is the mechanism of action of damage to the nervous system of organic lead compounds. The effect of organic lead compounds upon the membranes of the nervous system is being studied:

1. Membrane components that are being investigated consist of relatively widespread parameters including Na^+K^+ATPase, and peroxidative activity within membranes. Membrane fluidity will be studied using fluorescent probes. Regions of treated animals that are emphasized include frontal cortex and hippocampus where morphological damage is most apparent following exposure to lead.

2. Metabolic studies will reveal abnormalities of regional blood flow, glucose consumption, the ability to concentrate amino acids, and the integrity of the blood brain barrier.

3. The disruption of the normal role of calcium and zinc by lead may in part account for the effects of this toxicant on membrane structure, energy generation, and neurotransmission. Thus several enzymes and transport processes involving these essential metals are being assayed. These include phosphodiesterase, superoxide dismutase, glutamate dehydrogenase, and the synaptosomal uptake of calcium.

Principal Investigator and All Other Personnel Engaged on the Project:

S. C. Bondy Head, Neurochemistry Workgroup LBNT NIEHS

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-radioactive competing chemical.

Other laboratory methods include a series of enzyme assays, the capacity to measure nucleic acids, lipid peroxidation, cerebral blood flow, and regional glucose consumption.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Development of Techniques

A series of metal-dependent enzymes can now be measured including glutamate dehydrogenase, superoxide dismutase, phosphodiesterase.

Recent high affinity binding methods that have been developed include the ability to assay the Na^+K^+ATPase binding site for ouabain and the calcium channel binding site. It is planned to develop assays for a copper dependent enzyme (tyrosinase) and a myelin-related enzyme (2',3' cyclic nucleotide phosphodiesterase). A fluormetric probe will be used so that the fluidity of membranes can be estimated. Finally, the routine estimation of trace metals in brain tissue is planned, using anode-stripping voltametry.

B. Studies on Organic Metal Compounds

Treatment of adult male rats with triethyl lead chloride (6.9 mg/kg by intraperitoneal injection) has no detectable effect on hippocampal levels of the calcium-dependent 3'5' cyclic nucleotide phosphodiesterase or the binding characteristics of labeled calcium to hippocampal membranes. However, seven days after injection, the calcium transport channels in membranes appear to be reduced, as judged by binding of the antagonist nitrendipine.

At this time point, the zinc-dependent enzyme glutamate dehydrogenase is also depressed in the hippocampus. However, the zinc and copper dependent superoxide dismutase levels are elevated. This elevation is reversed 21 days after administration of TEL (triethyl lead chloride).

Neurotransmitter and other receptor levels have also been assayed at this dose of TEL. Benzodiazepine binding is decreased in the hippocampus, but not the cortex or striatum within one day of treatment and this effect is lost

three weeks later. Muscarinic cholinergic and GABA receptors are not altered in the first two weeks after TEL dosing, but at four weeks both of these binding species appear depressed in the frontal cortex and elevated in the hippocampus of treated rats. Future work will include:

1. Survey of a copper-dependent enzyme (tyrosinase) and another zinc dependent enzyme (leucine aminopeptidase) in brain regions of TEL treated animals.

2. Evaluation of the state of myelination of treated rats by assay of 2'3' cyclic nucleotide phosphohydrolase.

3. Analysis of other neurotransmitter receptors including glutamate which plays an important role in neuronal excitatory pathways, and the opiate receptor which may play a role in the transient analgesia seen in TEL-treated rats.

4. Measurement of membrane integrity by study of glucose uptake, blood brain barrier, and membrane fluidity. Preliminary work with another organometal (trimethyl tin) suggests the glucose uptake capacity of tissues may be modulated by these compounds.

C. Related Studies

The potential of the retina as a target for neurotoxic studies is being evaluated. The transmitter receptor population of both neural retina and the pigment epithelium has been described and the modulation of such receptors by environmental and toxic factors has been studied. The supply of nutrients to the retina under various states of excitation has also been examined. Direct intraocular injection of toxicants allows the measurement of damage to a precise neuronal population without metabolic modification of the toxic agent by the liver.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

This work aims at a description in biochemical terms of the series of events occurring in TEL-treated rats leading to abnormal behavior and other neurotoxic signs. The approach includes both nervous system-specific parameters and general membrane phenomena. Organometals are common in the environment and the means by which they cause neurotoxicity is a significant problem. Furthermore, some of the findings may be relevant to the neurotoxicity of the even more widespread inorganic forms of lead.

PUBLICATIONS

Ali, S. F. Hong, J. S., and Bondy, S. C.: Alterations in retinal neuropeptides of the chick by kainic acid and acrylamide. Brain Res. (Accepted).

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Ali, S. F., Hong, J. S., Uphouse, L. L., Wilson, W. E., and Bondy, S. C.: Effects of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. Arch. Toxicol. 52: 35-43, 1983.

Bondy, S. C.: Neurotransmitter binding interactions as a screen for neurotoxicity. In Vernadakis, A. and Prasad, K. N. (eds.): Mechanisms of Neurotoxic Substances. Raven Press, New York, 1982, pp. 25-50.

Bondy, S. C., Ali, S. F., Hong, J. S., Wilson, W. E., Fletcher, T., and Chader, G.: Neurotransmitter-related features of the retinal pigment epithelium. Neurochem. Internatl. (Accepted).

Bondy, S. C., Werdel, J., Fletcher, R. T., and Chader, G. J.: Retinal pigment epithelium contains a distinctive strychnine-binding receptor. Neurochem. Res. 7(12): 1445-52, 1982.

Hung, C. R., Hong, J. S., and Bondy, S. C.: Lack of asymmetrical distribution of receptor binding sites and neurally active peptides within rat brain. Neuroscience 7: 2295-98, 1982.

Hung, C. R., Hong, J. S., and Bondy, S. C.: The prevention of an artifact in receptor binding assay by an improved technique. Life Sci. 30: 1713-20, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90031-02 LBNT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Assessment of Neurophysiological Effects of Organometals

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)
(Name, title, laboratory, and institute affiliation)
Clifford L. Mitchell Chief LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Clifford L. Mitchell	Laboratory Chief	LBNT	NIEHS
H. Scott Swartzwelder	Staff Fellow	LBNT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The initial approach was to determine the single dose, 28 day LD50 by the subcutaneous (SC) route for trimethyl lead (TML), triethyl lead (TEL), trimethyl tin (TMT), and triethyl tin (TET) in Fischer 344 rats. This was done in order to select a common basis for the selection of doses of those agents in future studies. Subsequently, the ability of 1/4, 1/2, or 3/4 of the LD50 for each of the compounds to exacerbate convulsive activity elicited by pentylenetetrazol (PTZ) is examined. Times after toxicant administration most commonly studied are 1, 7, 14, 21, and 28 days. The purpose of these studies is to establish the doses and time intervals to be utilized for subsequent electrophysiological and neurochemical investigations.

The next step is to investigate the integrity of the limbic system by afterdischarge, kindling, and evoked potential techniques utilizing the dosages and time intervals determined from the preceeding studies. Comparisons are then made with other systems (e.g., auditory, visual somatosensory) to ascertain the relative specificity of any effect observed.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of TEL, TML and TMT on PTZ-induced seizures have been investigated. PTZ was administered interperitoneally (I.P.) in a dose of 35 mg/kg. This dose reliably produced a mild array of epileptiform signs in the normal Fischer 344 rat. After the animal was treated with PTZ, it was immediately placed in a sound-attenuating chamber and observed for a period of 10 minutes. Any epileptiform signs and their latency were recorded. Two designs have been used to date. The first is a repeated measures design wherein the rats are treated with a range of dosages of any organometal or with the saline vehicle, and then are tested at 1, 7, 14, 21 and 28 days after this acute treatment. This design allows for the assessment of seizure susceptibility and the development of kindling among the rats. The second design is a nonrepeated measures one. In these studies the animals are given injections of the metals or saline at 28, 21, 14 7 or 1 day prior to the single PTZ challenge. Thus, each animal is treated only once with the PTZ and the possible confound of kindling development is controlled.

TEL in single doses of 2.63, 5.25 and 7.88 mg/kg S.C. increased the sensitivity to PTZ. This increase was apparent 1 day post dosing and reached a maximal severity by day 14 which was maintained throughout the testing sequence. There was a transient loss of body weight in animals receiving the two higher doses with recovery generally occurring by day 14.

The effect of single doses of TML on PTZ-induced seizures is less clear than those for TEL. In general, the two lower doses (8 and 17 mg/kg S.C.) appears to produce a slight increase in seizure susceptibility which is observable from days 14-28 post TML dose. The highest dose (23 mg/kg) has produced either no effect or a decrease in seizure susceptibility at these

time points. Only the highest dose of TML produced a loss of body weight. This loss was apparent by day 7 and slowly returned toward normal over the 28 day period.

No effect on PTZ-induced seizures was observed with TMT in single doses of 1.5, 3 and 6 mg/kg S.C. over the 28 day period. However, a marked loss of body weight occurred during the first 7 days after the high dose, after which the animals again began to gain weight.

If the working hypothesis appears to be correct that the limbic system is most vulnerable to perturbation by at least some of these organometals, studies will be initiated to further test this hypothesis, to attempt to more clearly determine the site(s) within the limbic system which are most affected and to determine the molecular basis for the effects(s). The following are examples of possible approaches.

1. Comparison of intra-limbic system effects of microinjection of Kainic acid, AF64A (an agent which selectively lesions cholinergic neurons) with similar injections of the organometals using the electrophysiological procedures mentioned above.

2. Use of the in vitro hippocampal slice technique to compare single or multiple unit potentials from selected portions of the hippocampus in treated and control animals. This can be useful both for localizing effects of the organometals within the hippocampus and studying mechanisms. The latter can be done by adding neuropeptides, neurotransmitters or trace metals to the bath to study how these alter the unit potentials.

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 site(s) and mechanism(s) by which these agents produce their neurotoxicity. The results of this research will provide significant data on where within the brain and how these agents affect neural tissue. This information will be useful in the development and assessment of logical strategies for the detection of toxicity and/or treatment of exposed individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-90033-01 LBNT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Milk Bombesin

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lawrence H. Lazarus Research Chemist LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The existence of peptide containing immunoreactivity to bombesin has been identified in the whey of bovine milk. This is a new, previously unidentified peptide in mammalian tissue that is larger than the heptacosal gastrin releasing peptide (GRP) from porcine non-antral mucosa and the amphibian bombesin tetradecapeptide. This "milk bombesin" was separated from GRP by both gel filtration and reverse-phase HPLC. However, both porcine GRP and chicken GRP (mammalian analogue of alytesin, another bombesin-related peptide) produced nonparallel displacement curves, whereas "milk bombesin" was always parallel. Cleavage by trypsin produced a smaller immunoreactive peptide; alkylation of a urea/DTT treated sample of "milk bombesin" had no effect on its elution pattern on gel chromatography; thus, it is neither an aggreate of GRP or bombesin or both, nor combined with any extraneous milk protein. High speed centrifugation proved that the peptide was not associated with cell debris, bacterial or viral particles, or milk lipids. These studies provide evidence for the presence of another unique form of bombesin in mammalian tissue.

Principal Investigator and All Other Personnel Engaged on the Project:

L. H. Lazarus	Research Chemist	LBNT	NIEHS
W. E. Wilson	Research Chemist	LBNT	NIEHS
B. J. Irons	Biological Technician	LBNT	NIEHS
O. Hernandez	Research Chemistry	LBNT	NIEHS
M. Tulley	Director, Milk Bank	Wake County Memorial Hospital, Raleigh, NC	

PROJECT DESCRIPTION

METHODS EMPLOYED: Instant dry, nonfat milk will be suspended in water, acidified and clarified. The addition of alcohol to 90% precipitates bombesin immunoreactivity which is then resolubilized in 30-50% ethanol. This material is chromatographed on a Bio-Gel P-4 column and the peak of immunoreactivity is absorbed onto reverse-phase resins. This last step is recycled several times until a single peak of U.V. absorbance corresponds to the RIA peak.

MAJOR FINDINGS AND PROPOSED COURSE: The presence of a peptide in milk with immunological cross-reactivity with bombesin is a major finding indeed. It is known that the amphibian peptide bombesin can bring about the release of gastric acid in humans at very lower levels; the amount of immunoreactivity in a glass of milk (>220 ng) is more than sufficient to produce severe abdominal pain to ulcer patients. In addition, numerous gastrointestinal peptides that are found in elevated plasma levels occur upon the ingestion of milk by neonates are the same that are affected by bombesin infusion studies. Thus, it seems that a substance in milk, presumably "milk bombesin", may be responsible for these changes in gut performance. The proposed venue for future research with "milk bombesin" entails several facets: (1) purification of the peptide from milk and a determination of its sequence; (2) an assessment of its quantity from human breast milk at various times postpartum of preterm and full term pregnancies and different times during a feeding period; and (3) the effects of neurotoxicants on its level in lactating animal models; and (4) determine the tissue of origin of this new peptide. Each of these major facets of the work can be expanded upon, which depends, in turn, on the degree of success that they provide. The most promising issue to date is the rapidity in defining a purification scheme and the screening of human breast milk samples.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The presence of a unique peptide in milk that may regulate numerous peptide hormones in the gastrointestinal tract as well as gastric acid is a remarkable observation. This peptide may answer questions such as why milk cannot be used in ulcer therapy, and why certain human milks in the milk bank are unfit for use for consumption by infants. An epidemological survey of toxicants in nursing mothers reveal a correlation between an environmental pollutant a milkbourne peptide. Various animal model systems may be applicable to these potential and far-reaching studies.

NEW YORK UNIVERSITY MEDICAL CENTER - New York, NY 10016
(NIH-NO1-ES-2-5017)

TITLE: Use of Homecage Behaviors to Screen for Potential Toxicity of Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Hugh L. Evans, Ph.D.

PROJECT OFFICER (NIEHS): Hugh A. Tilson, Ph.D., Head
Neurobehavioral Workgroup, LBNT

DATE CONTRACT INITIATED: May 25, 1982

CURRENT ANNUAL LEVEL: $113,295

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this research program is to devise and validate methods to assess the effects of neurotoxicants using naturally occurring behaviors as the endpoints.

METHODS EMPLOYED:

The animals used in this project will be male, albino rats of the Fischer-344 strain weighing approximately 200 g at the beginning of the study. The animals will be housed in quarters having a constant 12 hr light-dark cycle (i.e., light 7 a.m. to 7 p.m.), temperature (21 ± 2°C) and relative humidity (50 ± 10%). The contractor shall proceed with the research in two steps.

(1) In the first phase of this program, the contractor will construct a home-cage monitoring system that allows for the continuous monitoring of food and water ingestion (e.g., amount ingested, time spent eating, meal size, meal patterning, etc.) and spontaneous motor activity (i.e., frequency and patterning of horizontally-directed activity and rearing). The entire system will be compact (i.e., about the size of a standard animal housing rack) and will be capable of testing up to 30-50 animals at a time (i.e., 2-3 experimental groups and at least one control group, 10 animals per group).

The system includes all hardware (i.e., behavioral monitoring apparatus and interfacing, and software (i.e., recording behavioral events and subsequent transformation into meaingful data for statistical interpretation). Phase 1 should require approximately 6-12 months for completion; this phase should also include a 28 day pilot study with several untreated animals (N=10-20) to domonstrate the reliability of the hardware and software.

(2) In the second phase, the contractor will conduct testing of at least six chemicals, including acrylamide, chlordecone (Kepone), arsenic trioxide, tetraethyl tin triethyl lead, and methylmercury hydroxide. This step will consist of dosing rats by gavage 5 days per week for 90 days. Homecage behaviors will be monitored for at least 5 days prior to dosing, during the dosing period, and for six weeks after cessation of dosing. At the end of dosing, half of the animals in each treatment group will be sacrificed and the amount of the chemical accumulated in the body will be determined. Body burdens will be determined in the remaining animals six weeks after cessation of dosing. It is anticipated that at least three compounds can be studied per year.

MAJOR FINDINGS AND PROPOSED COURSE:

The contractor has completed the first phase of the program. A homecage monitoring system has been constructed to measure continuously food and water ingestion and spontaneous motor activity. Pilot work is now underway and phase 2, assessment of chemicals, will begin soon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

One of the earliest indicators of toxicity is a change in body weight. The system being developed by this contract will permit the quantification of changes in ingestive behaviors and motor activity that may precede body weight alterations. The use of behavioral indices of toxicity may prove to be an early warning for the detection of toxicity, as well as provide clues to the discovery of how toxicants interfere with behavioral processes controlling food and water intake.

NORTH CAROLINA STATE UNIVERSITY
(NIH-NO1-ES-2-5019)

TITLE: Research Support to Investigate Teratogenic Effects of Environmental Agents Using Avian Species

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Behavioral and Neurological Toxicology

DATE CONTRACT INITIATED: August 1, 1982

CURRENT FUNDING LEVEL: $134,980

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the contract are to provide housing and maintenance services for care of research animals (Japanese quail) and to provide support staff necessary to conduct biological tests on birds which have been exposed to environmental agents.

METHODS EMPLOYED:

The contract will provide a special breeding colony for adequate supply of fertilized Japanese quail eggs, brooding facilities for housing and care of baby quail, and housing and maintenance of experimental Japanese quail throughout the duration of experiments. The contractor will also provide support personnel, materials, supplies, facilities and equipment for conduct of biological experiments. The support personnel will include a full-time professional level researcher, two full-time technical support persons, and two part-time caretaking and maintenance support persons. Research protocols will be developed by the staff of the Laboratory of Behavioral and Neurological Toxicology (LBNT) of NIEHS with the participation of the professionals provided by the contractor. The research projects will then be conducted by the personnel provided by the contractor with consultation and assistance of the staff at NIEHS.

MAJOR FINDINGS AND PROPOSED COURSE:

Eight research protocols have been written by the staff of NIEHS and are in progress at this time. Six groups of fertilized eggs have been exposed to microwaves and hematologic changes following antigen challenge is being determined in 5 and 12 week old quail. Baseline data on the immunological response of Japanese quail following injection of Chukar partridge red blood cells as an antigen have been obtained, and this technique will be used in the microwave exposed birds. A method for counting total leukocyte numbers in Japanese quail was developed using an electronic particle counter.

Studies to determine LD50 and day 2 LD50 for diethylstilbestrol (DES) have been completed. Injections of corn oil into the albumin through the small end of the egg were most effective for the administration of DES into quail embryos. Corn oil depressed hatchability below that of non-injected controls. DES injection to 31 µg/egg depressed hatchability below that of corn oil injected controls. DES at a dose of 31 µg/egg produced 50% mortality in chicks at 2 days after hatch. However, 90% mortality was reached by 3 day post-hatch. DES injected to 1.9 and 0.9 µg/egg produced 50% and 10% day 2 mortality respectively. In

another trial 0.9 µg/egg of DES produced 50% mortality by day 3 post-hatch. The apparent LD50 for chick liveability was 0.9 µg/egg. Females tended toward a higher mortality than males. A DES level of 450 ng/egg is currently being tested along with a corn oil source evaluation and neurobehavioral effect of 0.9 µg DES/egg.

The proposed direction of research to determine the effects of microwave exposure during embryogeny will include studies to evaluate hematologic changes following antigen challenge in juvenile and adult quail. In addition studies to investigate the effects of microwave exposure during embryogeny on hematologic and biochemical response of juvenile and adult Japanese quail to acute hemorrhagic stress will be conducted. Research to determine the effects of injection of DES into fertilized Japanese quail eggs will continue. Dose level of 0.9 µg/egg and 0.45 µg/egg will be used to evaluate the developmental, behavioral, and sexual effects of DES. Studies to determine LD50 and day 2 LD50 of kepone will be carried out in order to select effective doses for future behavioral studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The results of this research will increase our knowledge of the biological effects of microwave radiation and chemical agents. The use of the avian embryo as a test system provides the capability of studying the direct effects of environmenta agents on development without maternal complications.

LABORATORY OF GENETICS

LABORATORY OF GENETICS
Summary Statement

During the past year arrangements to merge the Laboratories of Animal Genetics, Molecular Genetics and Biochemical Genetics into a single unit were completed. The new Laboratory of Genetics now consists of three sections: Eukaryotic Gene Structure, Mutagenesis and Mammalian Mutagenesis.

Eukaryotic Gene Structure: The research in this section is focused on studies of the organization, function and regulation of eukaryotic genes and on the genetic architecture of eukaryotic populations. The objectives are to define in precise terms the fundamental mechanisms of gene action and how genes are developmentally regulated in order to understand the ways in which mutational changes modify gene action, and to define at the molecular level the types and amounts of genetic variability in populations of eukaryotic organisms.

The white locus in Drosophila melanogaster with its large number of mutant alleles provides a good model system for examining the organization of eukaryotic genes and how they are regulated. Most of the spontaneous mutations of this locus are the result of insertions of transposable sequences of DNA of unknown origin and function. One such mutant (w^{zm}) upsets aspects of the regulation of gene and is also somewhat mutable. It and its derivatives, also associated with insertions or deletions of mobile sequences of DNA, are being characterized by Dr. Judd. It is of considerable interest to learn how the gene has been modified in some mutant alleles to cause them to developmentally switch from a clonal, autonomous expression to a nonclonal nonautonomous mode. Other white locus studies indicate that transposable elements are sometimes responsible for creating duplication deficiency chromosomes through pairing and exchange by copies of the element at different sites in the chromosome. This is possibly part of a general mechanism by which these repeated insertion sequences are involved in chromosomal rearrangement events.

The cut locus, studied by Dr. Jack, is a model system of great interest because some of the mutant alleles upset function of the locus in specific tissues but allow what appears to be normal function in others. Again the spontaneous mutations are either insertions of retrovirus-like sequences into the locus or deletions of portions of it. A most unusual phenomenon discovered by Dr. J. Lim involves the destabilization of one X chromosome by another in such a way that the sex-linked cut mutant (ct^6), containing a transposable element, is transmitted to offspring without being expressed in the soma of the hemizygous F_2 male. The molecular analyses by Dr. Jack show that the cryptically transmitted allele has the molecular characteristics of ct^6 derived from the unstable chromosome. Efforts are underway to determine how the transmission of the mutation through the germline is accomplished while in the same individual the normal phenotype of the locus is expressed in somatic tissues.

Still another aspect of the role that transposable elements appear to have assumed in the mutation and regulation of eukaryotic genes is being investigated by Drs. Voelker, Searles and Chang. The suppressor of sable

(su(s)) mutation has the effect of suppressing selected spontaneous mutations at a number of loci. Transposons are clearly implicated as causal agents for at least some of these mutations. The su(s) locus has been cloned into recombinant DNA molecules and the vermilion (v) locus, which has several suppressible alleles, is also amenable to cloning through the "transposon tagging" technique. The molecular details of how suppression is accomplished should be approachable using these tools.

Dr. Searles and Voelker continue the collaboration with Dr. Greenleaf at Duke University on the analysis of the genes encoding RNA polymerase II. The gene encoding the 215,000 dalton subunit of the complex has been cloned. Mutant forms and revertants are being analyzed to determine how this enzyme complex is assembled and regulated. It is hoped that understanding how the synthesis of this complex is regulated will provide clues about the genes that encode other subunits of the complex.

Dr. Boswell's study of germ cell determination is aimed at understanding the nature of and genetic control of developmental determinants, the way in which determinants exert their effects and how the determined state of specific cell types is maintained. Mutants that impose a maternal effect on germ cell development present excellent material for examining these aspects of development. Grandchildless mutant strains of Drosophila produce offspring in which germ cells fail to develop and thus are sterile. To understand the factors involved in the determination and these cells, the nature of mutant genes is being studied from the molecular, genetic and developmental aspects.

Dr. Li's studies concern primarily the genes encoding mammalian lactate dehydrogenase isozymes. Several of the LDH genes from the mouse and human have been cloned and one encoding LDH-A has been sequenced. The complete amino acid sequences for mouse and rat LDH-C have been determined and the three dimensional structures have been visualized by computer program. This information supports work on the immunological properties of these enzymes and has speeded the molecular analysis of the cloned DNA segments. The objectives are to elucidate the mechanisms of tissue-specific expression of the LDH subunits and to examine the evolutionary relationships that exist between isozymes and between enzyme forms found in different vertebrate species.

In the molecular population genetics group, a major effort is being made to determine the nature and amount of genetic variation that exists at the DNA nucleotide level. Drs. Langley and Aquadro have analyzed the region around the alcohol dehydrogenase (Adh) locus in D. melanogaster by restriction enzyme digestion. They have determined that insertion/deletion differences around this gene are rather common. The types, frequencies and distributions of these insertion and deletions suggest that they produce small deleterious effects. Substantially fewer insertions and deletions were found in the regions flanking the dopa decarboxylase gene. Data such as these are important for establishing base line levels of variation and determining what types of variability can be tolerated in and around eukaryotic gene sequences.

Mammalian Mutagenesis: The primary objective of the program in the Mammalian Mutagenesis Section is to understand the mechanisms of mutations,

the differential sensitivity of various loci to mutagenesis, the relationship of mutations to the organization of the genome, and the phenotypical consequences of mutations. Mutations may arise from a series of different mechanisms such as a direct chemical change in the DNA caused by reaction of the agent with the DNA, its precursors or the enzymes involved in DNA replication or repair, or through more genetically dependent mechanisms including gene conversions or movement of transposable elements. The relative roles that these mutational processes play in the production of spontaneous and induced mutations is not known. Drs. Skow, Lewis and Johnson are in the process of analyzing, on the gene level, a collection of germinal mutations produced by various chemicals in the course of experiments using the biochemical specific locus test. The genes to be analyzed encode glucose isomerase (GPI), malic enzyme (MOD-1), a peptidase (PEP-3), hemoglobin A (HBA), and hemoglobin B (HBB). Multiple independently induced or spontaneous variants for each of the genes have been accumulated. DNA probes for these genes exist, or are in the process of being produced making possible the molecular analysis of normal and mutant alleles. The number and type of genes in which mutations can be detected are expanding. Special emphasis is given to loci that model human heritable traits such as cataracts; also important are those that are polymorphic in the human population. Screening for modifications of the lens proteins in the biochemical specific locus test in mice will add another 13 loci, bringing the total to approximately 30 loci in the test system.

Another type of mutation that is little understood in mammals is that which affects morphometric characters. A mutation detection system using dominant skeletal mutations has been described by other laboratories. Most of these studies were done with strains of animals of ill-defined genetic background which may cause difficulties in interpreting the data. A similar system is now under development in the Mammalian Mutagenesis Section using morphometric measurements of various mouse bones with shapes that are strongly genetically determined. The data obtained recently have shown no difference between offspring from ENU-treated males and from untreated males. The research continues increasing the strength of the statistical analysis and the sample size of the screened population.

Development of systems for detection of mutations in cells from the exposed individual (animal or human) would be extremely important for understanding the genetic damage caused by exposure to mutagenic agents and for elucidating mutation mechanisms in the mammal. A method investigated by Dr. Malling for detection of mutations in mouse sperm based on the antigenic differences between mouse LDH-C and rat LDH-C has encountered problems. Presumptive mutations were defined as those mouse sperm that reacted with an antibody specific to rat LDH-C. In previous studies crude agents were used for producing and isolating the monospecific IgG fraction. In recent studies we have used pure agents and did not detect any stained mouse sperm. Recently, by using less absorbed antibodies, a number of stained mouse sperm were detected. The frequency varied so much with the absorption procedure that no reproducible mutation data were obtained. The experience gained from the LDH-C system will be very useful for selection of the new markers to be used. The following factors may all be important for selection of the markers: mutability of the site, ability to produce pure monospecific antibodies, genetic construction of cells mimicking the mutant cell, and knowledge of the precise site for mutation. MOD-1 and

GPI-1 are presently under investigation for possible use as markers for single cell mutation systems. Many of the markers that are available are expressed in both somatic and germinal tissues which permits comparison between mutational events occurring in these tissues. By selecting markers for study of mutagenesis in single cells in vivo, which are also used for study of induction of transmissible mutations, the pattern of mutagenesis in the two different sets of mutants can be compared.

Mutagenesis: The objective of the Mutagenesis Section of the Laboratory of Genetics is to elucidate the molecular mechanisms of mutagenesis. Both genetic and biochemical techniques are employed by members of the group in an effort to define the parameters involved in maintenance of the genome. The detailed description sought by this group will serve as a basis for understanding and predicting the genetic effects of environmental chemicals.

Mutagenesis research is conducted in the laboratories of five lead scientists under the overall direction of Dr. John W. Drake, Head of the Mutagenesis Section.

In Dr. Drake's laboratory the bacteriophage T4 is used as a model system. Studies of "error-prone repair" in T4 have demonstrated that a number of published claims about the mechanism of UV mutagenesis in T4 are suspect because of an experimental artifact. These studies also resulted in the recovery of a strain bearing a mutation in a putative T4 DNA repair gene (uvsZ) and another strain whose UV resistance is greater than that of the wild type; further analyses of these new mutants will contribute to a molecular description of error-prone repair in T4. Additionally, to aid in describing mutations in the T4 rII region at the level of DNA sequence, methods are being developed to enable sequencing of specific mutants without prior cloning of each individual mutant fragment.

Dr. Lynn S. Ripley's group is also using T4 as a model system. The focus of their investigation is the role of T4 DNA polymerase in the generation of frameshift mutations. Genetic analysis of frameshift mutations occurring in the rII region has shown that each of the six polymerase alleles thus far tested produces a unique mutational spectrum. These genetic approaches have been coupled with DNA sequence analysis to identify more precisely the nature of the frameshifts and to permit the formulation of more detailed models of the mechanisms resulting in frameshift mutations. Novel M13-T4rII hybrids have now been constructed which will simplify cloning of T4 frameshift sequences. These hybrids will facilitate sequencing of mutations produced both in vivo and in vitro and can then be used to develop new DNA substrates for further probing the mechanisms of frameshift and deletion mutagenesis.

Analysis of new T4 rII frameshift mutations, observations made in the E. coli lacI system, and examination of a large body of information in the literature prompted Drs. Ripley and Barry W. Glickman to formulate models involving DNA secondary structures as intermediates in the formation of several types of mutations. Sequence analysis of T4 rII frameshift mutations has now established that secondary structures are likely intermediates in the formation of a significant class of frameshifts as predicted by these models. Accumulation of further sequence data from T4

and from the lacI system is in progress and will permit a more accurate assessment of the involvement of secondary structures as intermediates in mutational events.

The group led by Dr. Michael R. Volkert uses E. coli genetic procedures to investigate the processes involved in the cellular metabolism of damaged DNA. Current projects investigate genes involved in the SOS response, and any others whose products are induced by alkylating agents. Specific approaches include: (1) selection and characterization of suppressors of recF, a gene whose product is involved in metabolism of both normal and damaged DNA; (2) analysis of the physiological effects of recF mutations by studing them in strains which also carry the recA441 mutation (in which the SOS response is induced at 42 C); (3) measurement of UV-reactivation and mutagenesis of UV-damaged X174 DNA in the presence of various combinations of mutations in genes involved in the SOS response, particularly recA441 and umuC; and (4) selection and characterization of mutants deficient in the repair of alkylated DNA.

E. coli genetic analysis is also employed by Dr. Glickman's laboratory to examine the mechanism of mismatch repair and the effects of mutagen dose on mutational specificity. Analysis of the effects of mismatch repair on errors induced by 2-aminopurine has shown that mismatch repair is required to effectively correct base analog-induced errors, that a new hot spot for 2-aminopurine mutagenesis is found in mismatch repair-deficient strains, and that mismatch repair-defective strains show increased levels of mutation following induction of the SOS response. The effect of mutagen dose on mutational specificity has been determined by characterizing the spectra of mutations produced in the E. coli lacI gene by a number of potentially mutagenic agents including ionizing and UV radiations, alkylating agents, hydroxylamine, bisulfite, and thymine deprivation. This characterization permits analyses of different cellular repair capacities, providing a basis for mechanistic models of mutagenesis and for more accurate risk assessment. For example, studies of mutation induced by UV irradiation suggest that Pyr-Pyr 6-4 lesions are primary premutagenic lesions in double-stranded DNA, while Pyr-Pyr cyclobutane dimers appear to be premutagenic lesions in F' transfer experiments where the transferred DNA is single-stranded.

In a major new project in Dr. Glickman's group, recombinant DNA techniques are being used by Dr. Richard A. Zakour to investigate mutagenesis in mammalian cells. Shuttle vectors have been constructed that can be selected both in mammalian cells and in E. coli. These will be introduced into mammalian cells and subjected to various mutagenic treatments. The mutated vectors will then be transferred to E. coli and subjected to genetic and DNA sequence analyses. The project is expected to illuminate mechanisms of mutation induction in mammals as well as to improve estimates of risk posed to humans by potential environmental hazards.

Dr. Thomas A. Kunkel's group uses as models the well defined E. coli single-stranded DNA bacteriophages X174am3 and M13mp2, which permit efficient identification and DNA sequence determination of mutations occurring under various conditions. Studies currently in progress include analyses of the mutagenic consequences of depurination (the loss of a purine base from DNA) and measurements of the fidelity of purified DNA polymerases.

Depurination has been shown to be highly mutagenic, to result primarily in base substitutions, and to be largely dependent on a functional error-prone repair system. In vitro analysis of the fidelity of DNA polymerases provides important information concerning the accuracy of the crucial enzymes involved in DNA synthesis.

In collaboration, the Kunkel and Glickman groups are examining the mutagenic consequences of defined lesions (e.g., apurinic sites) and of specific DNA sequences introduced into M13mp2 DNA. The introduction of specific lesions into this well defined DNA should provide information on the genetic consequences of a single type of DNA damage and overcome the problem of analyzing mutagenic effects of a wide spectrum of lesions induced by a single agent. The use of M13mp2 into which specific DNA sequences have been inserted should permit measurements of the influence of DNA sequence on the frequency and specificity of spontaneous and induced mutagenesis.

The group led by Dr. Akio Sugino, who is now working at the University of Georgia, investigated the mechanisms of DNA replication in eukaryotes using a yeast in vitro DNA replication system. Recent biochemical fractionation of the DNA-replicating cellular extracts has resulted in the purification and characterization of a DNA primase activity. Other experiments have focused on the role of DNA methylation in replication, and current results indicate that methylation of the template DNA at specific sites may play a significant role in regulation of DNA replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60098-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Lactate Dehydrogenase Isozyme Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Steven S.-L. Li Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

Computer Center Branch, Division of Computer Research and Technology
National Institutes of Health, Bethesda, Maryland

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure 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 *(Use standard unreduced type. Do not exceed the space provided.)*

The primary structures of LDH isozymes from mouse and rat testes as well as mouse muscle and heart have been determined by amino acid sequencing, and space-filling models of the LDH molecules have been constructed on a computer-graphic display system. Immunological properties of LDH-X isozymes from mouse and rat have been characterized, and their potential antigenic determinants have also been predicted.

Principal Investigator and All Other Personnel Engaged on the Project:

Steven S.-L. Li	Research Geneticist	LG	NIEHS
Farida S. Sharief	Biologist	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The purified lactate dehydrogenase proteins were 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. The space-filling models of LDH-X molecules have been constructed on a computer-graphic display system.

MAJOR FINDINGS AND PROPOSED COURSE: Both rat and mouse LDH-C_4 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-C_4 and 84% of rat LDH-C_4 have been determined. Ten percent of the 330 residues are different between these two LDH-C_4 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-C_4 molecule, may be involved in the unique antigenic determinants of the rat and the mouse LDH-C_4 isozymes. The primary structure of mouse LDH-A_4 isozyme has also been determined from sequence analyses of the protein and a cloned LDH-A cDNA. Sequence comparison among mammalian LDH isozymes clearly indicates that A_4 (muscle) and B_4 (heart) isozymes shows a closer evolutionary relationship to each other than to the C_4 (testis) isozyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The amino acid sequence differences among LDH-C_4 isozymes will be correlated with the antigenic properties of mammalian LDH isozymes. The chemical sequence of mouse LDH-C_4 will also be correlated with X-ray diffraction data in order to understand the structure-function relationship of this enzyme.

PUBLICATIONS

Pan, Y.-C.E., Sharief, F.S., Okabe, M., Huang, S., and Li, S.S.-L.: Amino acid sequence studies on lactate dehydrogenase C_4 isozymes from mouse and rat testes. J. Biol. Chem. 258: 7005-7016, 1983.

Li, S.S.-L., Feldmann, R.J., Okabe, M., and Pan, Y.-C.E.: Molecular features and immunological properties of lactate dehydrogenase C_4 isozymes from mouse and rat testes. J. Biol. Chem. 258: 7017-7028, 1983.

Li, S.S.-L., Fitch, W.M., Pan, Y.-C.E., and Sharief, F.S.: Evolutionary relationships of vertebrate lactate dehydrogenase isozymes, A_4 (muscle), B_4 (heart) and C_4 (testes). J. Biol. Chem. 258: 7029-7032, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60099-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organization-Regulation of Mammalian Lactate Dehydrogenase

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Steven S.-L. Li Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

Department of Molecular Biology, Northwestern University Medical School
Chicago, Illinois

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse LDH-A cDNA clones have been isolated, and the nucleotide sequence of recombinant plasmid pMLA73 has been determined. Several LDH genes have also isolated and partially characterized from mouse and human genomic libraries.

Principal Investigator and All Other Personnel Engaged on the Project:

Steven S.-L. Li	Research Geneticist	LG	NIEHS
Motoyuki Shimizu	Visiting Associate	LG	NIEHS
Hiroshi Tsujibo	Visiting Fellow	LG	NIEHS
Kiyohito Yagi	Visiting Fellow	LG	NIEHS
Farida S. Sharief	Biologist	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Recombinant plasmids of mouse LDH-A cDNA were isolated from mouse liver cDNA library by colony hybridization using the 500 bp probe of rat pRLD42. Several LDH genomic clones have been isolated from mouse and human libraries by plaque hybridization using mouse LDH-A cDNA probe.

MAJOR FINDINGS AND PROPOSED COURSE: Mouse LDH-A cDNA clones have been isolated, and 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. Several LDH genes have been isolated and partially characterized from mouse and human genomic libraries.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In mammals and birds the LDH-X(C_4) isozyme is found only in testes and spermatozoa, whereas the LDH-M(A_4) and LDH-H(B_4) 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

Shimizu, M., Tsujibo, H., Sharief, F.S., Tiano, H.F., Pan, Y.-C.E., Jungmann, R.A. and Li, S.S.-L.: Sequence analyses of cDNA and protein for mammalian lactate dehydrogenase isozymes. Fed. Proc. 42: 1970, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60109-05 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of DNA Replication in Eukaryotes

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Akio Sugino	Visiting Scientist	LGM	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An in vitro DNA replication system from yeast has been developed as a model system to investigate the mechanism of DNA replication in eukaryotes. Among the proteins identified as essential for DNA replication by this system are yeast DNA polymerase I and a single-stranded DNA binding protein. Recent biochemical fractionation of this system permitted the purification and characterization of a DNA primase activity. The potential role of DNA methylation in replication was investigated by measuring the extent of methylation of 2μm and ars-1 containing plasmid DNAs at different stages of the cell division cycle and by comparing as templates in the in vitro DNA replication system plasmid DNAs which were unmethylated or methylated by various methylases.

Principal Investigator and All Other Personnel Engaged on the Project:

Akio Sugino	Visiting Scientist	LGM	NIEHS
Frances W. Coleman	Staff Fellow	LGM	NIEHS
Akira Sakai	Visiting Fellow	LGM	NIEHS
Jennifer Motto	Biologist	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Column chromatography. Velocity sedimentation centrifugation. Sucrose density gradient centrifugation. Enzymatic DNA nucleotide sequencing procedures. Agarose and acrylamide gel electrophoresis. Restriction enzyme analysis of DNA. Southern transfer. DNA-DNA hybridization. ^{32}P-labeling of DNA by nick-translation. In vitro methylation.

MAJOR FINDINGS AND PROPOSED COURSE: A DNA primase activity was purified from the yeast Saccharomyces cerevisiae. Most of this activity is purified away from DNA polymerase I. It is required for in vitro replication of yeast 2 μm plasmid DNA and permits rNTP-dependent DNA synthesis by yeast DNA polymerase I on single-stranded circular viral templates and on synthetic poly $(dT)_n$. The primase activity resides in a single polypeptide with a molecular weight of 65,000 and is distinct from known RNA polymerases I, II and III. It synthesizes oligoribonucleotides which are mainly eight- or nine-mers. In the presence of primase and DNA polymerase I, DNA products of 300-500 nucleotides are synthesized on a single-stranded circular viral DNA. These DNAs are covalently linked to oligoribonucleotides at their 5' ends.

Further characterization of enzyme activities required for in vitro replication of yeast 2 μm plasmid DNA is in progress. While the primase activity just described was purified by biochemically fractionating crude cell extracts, the isolation of a set of mutants in yeast which are temperature sensitive for DNA replication is underway and will be employed to identify other DNA replication protein activities.

The relative cleavage patterns of the restriction enzymes Hpa II and Msp I were used to detect methylated 2 μm and ars-1 containing plasmid DNA sequences in yeast cells under varying growth conditions. In exponentially-growing cells, 2 μm and ars-1 plasmid DNAs were digested by Msp I but only partially digested by Hpa II, indicating that these DNAs are methylated at some positions in rapidly growing cells. In yeast cells arrested at G1 or G2, 2 μm and ars-1 plasmid DNAs were both digested to completion by Hpa II. Upon examination of the Hpa II cleavage maps of these plasmids it was found that most of the sites which are methylated in rapidly growing and not methylated in G1- or G2-arrested cells, occur at or near the origin of DNA replication.

Methylated and unmethylated 2 μm and ars-1 containing plasmid DNAs were compared as templates for the in vitro DNA replication system from yeast previously developed in this laboratory. It was determined that when these plasmid DNAs were

prepared from dam-3 (deoxyadenosine methylase$^-$) E. coli, DNA synthesis was 3-4-fold greater than DNA synthesis measured with the same templates prepared from dam$^+$ E. coli. If all CCGG sites in 2 μm and ars-1 plasmid DNAs were fully methylated by Haemophilus parainfluenza Hpa II methylase in vitro, the DNA is totally inactive in the in vitro DNA replication system under standard conditions. These results indicate that methylation of the DNA template at specific sites may play a significant role in regulation of DNA replication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough, detailed knowledge of the mechanism of DNA replication in eukaryotes will contribute to the understanding of the mechanism of action of various environmental mutagens.

PUBLICATIONS

Sugino, A. Sakai, A., Wilson-Coleman, F, Arendes, J., and K. C. Kim, In vitro reconstitution of yeast 2-μm plasmid DNA replication. ICN UCLA Symposia on Molecular and Cellular Biology: Mechanisms of DNA Replication and Recombination. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60111-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1982

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Role of Gene 43 DNA Polymerase in Frameshift Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lynn S. Ripley Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

Institute of Molec. Biology. IAMP
Univ. of Oregon NIH
Eugene, OR

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have already identified the T4 DNA polymerase as a major determinant in the frequency and specificity of spontaneous frameshift mutation, through the genetic analysis of mutation in the T4 rII genes. Our recent work has extended this analysis to the level of DNA sequencing of frameshift mutations. This more refined analysis confirms the general conclusions of the genetic analysis, but more importantly begins to identify the precise nature of the specificity changes promoted by mutant DNA polymerases. These specificity changes are of more than one kind, suggesting that several mechanisms of frameshift mutagenesis contribute to spontaneous frameshift mutation. Two novel classes of frameshift mutations resulting from previously undescribed misaligned DNA intermediates have been identified. Moreover, there are initial indications that primary DNA sequences may be important factors in defining frameshift mutation in the polymerase mutant tsL141. Construction of novel M13-T4rII hybrids has been completed. These hybrids should premit more efficient "genetic" cloning of T4 frameshift sequences, the ready genetic analysis of frameshift mutations produced in vitro, and the development of new DNA substrates for further probing specific mechanisms of frameshift and deletion mutations.

Principal Investigator and All Other Personnel Engaged on the Project:

Lynn S. Ripley	Senior Staff Fellow	LGM	NIEHS
Johan de Boer	Visiting Fellow	LGM	NIEHS
Alan Clark	Biologist	LGM	NIEHS
Kim Price	Summer Aid	LGM	NIEHS

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. Sequencing of T4 frameshift mutations has been accomplished by cloning Taq I fragments of T4 into M13, followed by dideoxy sequencing of the appropriate clones.

MAJOR FINDINGS AND PROPOSED COURSE: Complex frameshift mutations (DNA sequence changes producing not only a duplicated base or bases or a deleted base or bases, but also having associated base substitution changes, or insertions of sequences not obviously related by duplication to nearby DNA sequences) have been identified among the spontaneous rII frameshift mutants. One class of complex frameshift mutations was previously hypothesized to result from quasipalindromic DNA sequences on the basis of theoretical possibilities, and complex frameshift mutant sequences in yeast (Ripley, 1982). The model was predicted to be acting at quasipalindromic sequences in T4, a prediction confirmed in our recent studies. A dramatic example includes a frameshift mutation which results in a DNA sequence change influencing 5 non-contiguous base pairs in a DNA sequence extending for a distance of 10 base pairs. All of the changes arose in a single concerted event, and are precisely those changes predicted by the model previously proposed.

DNA sequences of mutants arising at a different rII frameshift site identify a novel frameshift mechanism, in which the mutation arises through the interaction of the mutant site with a DNA sequence at a distance (in this particular case, 256 base pairs distant) that bears substantial but imperfect homology with the mutant site. The mutational outcome is as though the sequence at the distant site directed the incorporation of bases at this imperfectly homologous site, converting the sequence at the mutant site to a sequence now perfectly homologous to the distant sequence. Further work in the area of complex frameshift mutation will involve further sequencing of mutants for a better identification of the frequency with which these mutations occur.

Complex mutations are generally considered infrequent. For example genetic analysis of mutational specificity depends on the much simpler assumption of single base changes, and these analyses might often be defeated in the face of frequenct multiple DNA sequence changes. Clearly the complex sequence changes we have seen, do not represent the "majority" of frameshift mutations, but they certainly represent a significant "minority". The identification of conditions

that specifically enhance this segment of mutation, or that permits its specific identification would greatly facilitate our study of parameters influencing the frequency and position of their occurrence. Although, the novel models for frameshift mutations that we have developed were undertaken as explanations for complex mutations where no other models provide successful explanations, these models also predict simple frameshift mutations and base substitutions. The wider applications of the predictions of these models to other DNA sequences including evolutionarily related DNA sequences is being undertaken in collaboration with B. W. Glickman and M. Zuker.

Extrapolation of mutational models involving quasipalindrome mediated frameshift and base substitution mutation have been extended to include models that successfully explain a substantial fraction of spontaneous deletion mutations. (Ripley and Glickman 1982; Glickman and Ripley, (submitted)). Our recent discovery of complex frameshift mutants in T4 that perfect, and partially perfect the quasipalindromes that template them, provide a particularly attractive substrate for examining quantitative and specificity aspects of this class of deletion mutation. The deletions are thought to arise by the perfect removal of entire quasipalindromes. This can be viewed as the removal of a DNA hairpin from DNA, although this may not be the mechanism by which the mutations occurs. The frameshift mutations create much longer and stronger potential DNA hairpins. The removal of the entire hairpin, relieves in this particular case, the frameshift, returning the frame to 0. Moreover, in this particular sequence, the deletion is not likely to create a mutant phenotype, and hence such deletion mutations are likely to occur among "revertants" of the frameshifts. Such frequencies can be easily measured, and genetic characterization of the revertants, can reveal the presence (or not) of the predicted deletions. Simple genetic constructions should be possible to perturb the hairpin, and hence potentially the frequency of such deletions.

Initial indication of frameshift specificity induced in the presence of the mutant polymerase tsL141 indicate a strong preference for the addition or deletion of a single A:T base pair. We intend to investigate this preference by sequencing a larger number of frameshifts produced by this particular mutant, particularly at genetic sites at which G:C base pair additions or deletions are frequent in the presence of other polymerases. If the correlation is confirmed, we will attempt to extend these studies into an in vitro characterization of tsL141 polymerase mediated mutation. The potential A:T preference is particularly provocative since this mutant polymerase allele, specifically reduces spontaneous A:T-site transitions, but does not influence the frequency of G:C-site transition mutations. Moreover, the failure to reduce G:C-site transitions is not predicted by popular models of base substitution fidelity and previous in vitro measurements of the nature of the defect of this enzyme. If a similar A:T and G:C site dichotomy exists for frameshifts, the enzymatic basis for the mutational specificity of this mutant polymerase may be addressable.

We intend to pursue the possibility that direct interaction of neighboring DNA sequences with DNA polymerase may in fact influence the frequency or specificity of frameshift mutation. It has recently been observed that during synthesis in vitro that certain primary DNA sequences appear to be associated with kinetic

pausing of the polymerase (E. Fairfield, unpublished results). We intend to use M13-T4 rII hybrids to determine whether T4 polymerase pauses are associated with spectra of mutation, in a way that suggests a causal relationship to the appearance or absence of mutation. The result of such studies provides a direct attempt to measure the potential of "neighboring base pairs" on DNA polymerization fidelity.

Studies of frameshift mutation at spontaneous "hot spots" sites for frameshift mutation in the rII genes led us to propose that frameshifts frequencies at these sites may be strongly influenced by the nearby initiation of Okazaki fragments (Ripley and Shoemaker, 1983). A direct test of this hypothesis will be undertaken, by creating nearby DNA sequences which will change the ability of the sites to initiate the fragments. The predictions are that creating new sites will increase mutation frequencies while deleting old sites will decrease mutation frequencies. The DNA sequence changes will be created by in vitro site-specific mutagenesis using M13-rII hybrid vectors. We have already developed recombination methods that permit the ready transfer of mutant sequences created in these vectors back into T4, by genetic recombination. The oligonucleotides required for creating the mutations are being synthesized.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Frameshift mechanisms are not understood. The identification of two novel mechanisms by which spontaneous mutations occur has already been achieved. These mechanisms depend upon the character of primary DNA sequences which is a universal feature of all DNA and this suggests the potential relevance of these mechanisms to all procaryotic and eucaryotic organisms. The mutations are mediated by DNA misalignments involving arrival DNA secondary structures. The relevance of alternative secondary structures to the mutational process and the enzymology by which the mutations are fixed will be of critical importance in predicting the frequency with which such mutations may contribute to mutation in procaryotic and eucaryotic systems. The complex frameshifts mediated by quasipalindromes are implicated in both T4 (a procaryote) and yeast (a eucaryote) by direct DNA sequencing of mutants. The identification of complex mutations, and the suggestion that they may not be infrequent, may have particularly profound effects on analysis of mutations and profound consequences in the discription of relatedness of DNA sequences. In both instances base changes are expected to arise as single events and to be relatively independent of the sequences in which they lie. The mutations that we are studying have neither of these qualities and thus their frequency with respect to mutations that do have these qualities will be an important questions for the future.

PUBLICATIONS

Ripley, L. S.: Model for the participated of quasipalindromic DNA sequences in frameshift mutation. Proc. Natl. Acad. Sci. USA 79: 4128-4132, 1982.

Ripley, L. S.: The specificity of infidelity of DNA polymerase. In Lawrence, C.W., Prakash, L.S., and Sherman, F. (Ed.): Induced Mutagenesis: Molecular Mechanisms and Their Implications for Environmental Protection. Plenum Press, NY, 1982, pp. 83-113.

Ripley, L. S., and Shoemaker, N.B.: Polymerase infidelity and frameshift mutation. In Lemontt, J.F., and Generoso, W.M. (Ed.): Molecular and Cellular Mechanisms of Mutagenesis. Plenum Press, NY, 1981, pp. 161-178.

Ripley, L. S., and Glickman, B.W.: The unique self-complementarity of palindromic sequences provides DNA structural intermediates for mutation. Cold Spring Harbor Symp. Quant. Biol. 47: 851-861, 1982.

Ripley, L. S., and Shoemaker, N.B.: A major role for DNA polymerase in frameshift mutation. Genetics. 103: 353-366, 1983.

Ripley, L. S., and Glickman, B.W.: DNA secondary structures and mutation. In Friedberg, E.C. and Bridges, B.R. (Ed.): Cellular Responses to DNA Damage, UCLA Symposia on Molecular and Cellular Biology, A New Series, Alan R. Liss, NY 11: (in press), 1983.

Ripley, L. S., Glickman, B. W., and Shoemaker, N.B.: Mutator versus antimutator activity of a T4 DNA polymerase distinguishes two different frameshifting mechanisms. Mol. Gen. Genet. 189: 113-117, 1983.

Glickman, B. W., and Ripley, L.S.: Structural intermediates of deletion mutagenesis: a role for palindromic DNA. PNAS (submitted), 1983.

Drake, J. W., Glickman, B. W., and Ripley, L. S.: De mutatis mutationum mutandis. Amer. Scientist (submitted), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60112-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanism of Mismatch Correction in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Barry W. Glickman	Expert	LGM	NIEHS

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 in inducible and that mismatch repair can avoid indirect mutagenesis due to the induction of SOS repair, and misincorporation errors due to miscoding lesions.

Principal Investigator and All Other Personnel Engaged on the Project:

Barry W. Glickman	Expert	LGM	NIEHS
Roel Schaaper	Visiting Fellow	LGM	NIEHS
Don Halderman	Biologist	LGM	NIEHS
Mary Skrzynski	Student Employee	LGM	NIEHS
Peter Dalldorf	Summer Graduate	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Our approach has relied on standard mutagenesis analysis as well as the analysis of mutational specificity in the lacI gene in E. coli strains carrying mismatch correction mutants. Recently we have used Mud:lac insertions to recover new mutants of mismatch repair.

MAJOR FINDINGS AND PROPOSED COURSE: 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 daugther DNA strands on the basis of DNA methylation levels, and key to discrimination being that parental DNA strands are fully methylated and daugther 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 incorporated, 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 effect of mismatch repair on errors induced by 2-aminopurine (2AP). We conclude that (1) Mismatch repair mutants show enhanced levels of 2AP-induced mutagenesis showing mismatch repair to effectively correct base analog induced errors. (2) The specificity of 2AP is for G:C → A:T transitions in the lacI system (A:T → G:C transitions cannot be measured). These occur primarily at sites proceeded by a G:C base pair. (3) The hotspots for 2AP found in the wild-type strain (Amber sites 6, 15 and 34) are also seen in mutH. However, a new hotspot at ochre 27 is found in the mutH strain. The sequence of this hotspot is 5'AAAAA(C)AG (the C → T site in parenthesis).

The role of mismatch repair in correcting errors made during SOS (untargeted?) mutagenesis was also examined. Mismatch repair was found to help avoid errors at non-dimer sites, i.e., *mutH, L, S* and *uvrE* mutant showed increased levels of mutation at non-dimer sites following UV-irradiation.

We have begun a study to investigate the control of mismatch repair and have isolated two Mud:*lac* insertions into genes "turned on" by 2-AP exposure. We hope by isolating Mud:*lac* fusions of the *mut* genes, to study 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 and DNA repair.

PUBLICATIONS

Glickman, B. W.: The mutational specificity of base analogue mutagenesis. In Genetic Consequences of Nucleotide Pool Imbalances as Part of the Conference - Genetics Consequences of Nucleotide Pool Imbalances. (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60113-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Mutagenesis in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Barry W. Glickman Expert LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	2.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Barry W. Glickman	Expert	LGM	NIEHS
Ronnie L. Dunn	Biologist	LGM	NIEHS
Donald Halderman	Biologist	LGM	NIEHS
Roel Schaaper	Visiting Fellow	LGM	NIEHS
Bernard Kunz	Visiting Fellow	LGM	NIEHS
Mary Skrzynski	Student Employee	LGM	NIEHS
Peter Dalldorf	Summer Graduate	LGM	NIEHS

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 developing a rapid DNA sequencing system for the determination of mutational specificity. This is being approached by homogenotization of lacI$^-$ mutants on the F' with an M13 derivative carrying lacI$^+$ lacZ$^-$.

MAJOR FINDINGS AND PROPOSED COURSE: 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. Photoreactivation studies showed that the amber 24 site photoreactivated readily while other hotspot sites photoreactivated poorly. Possibly more than one UV photoproduct may yield mutation. Evidence that Pyr-Pyr (6-4) lesions might cause mutation was obtained by studying the spectrum of UV-induced

mutation in a dcm⁻ mutant. As Pyr-Pyr (6-4) lesions do not form readily at sites of 5-methylation (thymines or 5-methyl cytosine), little mutagenesis is expected (and found) at C-5Me sequences. In a dcm⁻ strain where these sequences are not methylated, mutants are recovered following UV-irradiation. This suggests that Pyr-Pyr (6-4) photoproducts might be premutagenic lesions.

F' transfer experiments in which the donor strain was irradiated and photoreactivated prior to transfer suggest Pyr-Pyr cyclobutane dimers to be capable of being premutagenic lesions.

Ionizing radiation: 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 transitions (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. The biological effects of tritium decay was also examined. 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.

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, 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. Studies on the repair of alkylation damage reveal a role for dam, mutH, mutL and mutS in error-avoidance during EMS treatment. These mutants produced not only G:C → A:T transitions following EMS treatment, but also a significant fraction of transversions. Transversions were also recovered among EMS-induced mutants in a uvrB⁻ strain. These results indicate a role for both mismatch repair and excision repair in error-avoidance during EMS treatment.

Alk⁻ mutants, defective in the induced 3-methyladenine glycosylase showed a higher percentage of transversions following MMS treatment. Following MNNG treatment however, only G:C → A:T transitions were recovered. The source of the transversions following MMS treatment is currently under investigation.

Hydroxylamine: Treatment by HA produces a member of DNA adducts, among which is N -hydroxycytidine. This product is expected to cause G:C → A:T transitions. Indeed, following treatment by HA, G:C → A:T transitions were recovered. Transversions were also recovered. They arose in a recA-independent fashion and their origin is currently being investigated.

Bisulfite mutagenesis: Treatment by bisulfite was expected to cause G:C → A:T transitions. As bisulfite is highly active in single-stranded DNA, it seemed possible that bisulfite-induced hotspots might reveal sites of preferred single-strandedness or hairpin formation. However, bisulfite was not found to be mutagenic in the lacI system.

Thymine deprivation: Thymine deprivation from growing cells leads to nucleotide pool imbalances and cell death. A model involing both pool-imbalance induced errors and the lethal and mutagenic effects of attempts at DNA repair has been developed and partially confirmed. Both base substitution and frameshift mutations have been studied. Some errors appear to rise from the misincorporation of erroneous bases, others as a consequence of the repair of excision tracts made during the removal of uracil from the DNA in ung$^+$ but not ung$^-$ (uracil-glycosylase) strains.

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

Schaaper, R., and Glickman, B. W.: UV-induced mutagenesis: a prerequisite for a pyrimidine dimer at the target site Molec. Gen. Genet.

Shinoura, Y., Kato, T., and Glickman, B. W.: A rapid and simple method for the determination of base substitution and frameshift specificity of mutagens. Mutation Res. (in press).

Yukiko, S., Ise, T., Kako, T., and Glickman, B. W.: umuC-Mediated misrepair mutagenesis in Escherichia coli: extent and specificity of SOS mutagenesis. Mutation Res. (in press).

Kato, T., and Glickman, B. W.: Randomness of base substitution mutations induced by ionizing radiation. Science. (in press).

Ise, T., Kato, T., and Glickman, B. W.: Spectra of base substitution mutations induced in Escherichia coli by tritiated water and the decay of incorporated tritiated thymidine. Radiation Res. (in press).

Kunz, B. A.: Thymineless mutagenesis in bacteria. In Genetic consequences of nucleotide pool imbalances as part of the conference - Genetic consequences of nucleotide pool imbalances. (in press).

Kunz, B. A., and Glickman, B. W.: Absence of bisulfite mutagenesis in the lacI gene of Escherichia coli. Mutation Res. 119: 267-271, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60130-03 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation of E. coli Mutants Defective in Repair of Alkylated DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael R. Volkert Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. We are currently using the bacteriophage Mud(Aplac) as a mutagene and screening for insertions of this phage which express the lac gene after treatment with sublethal levels of alkylating agents have accumulate strains containing such insertions. These can be either 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.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael R. Volkert	Senior Staff Fellow	LGM	NIEHS
Dinh C. Nguyen	Biologist	LGM	NIEHS
K. Chris Beard	Summer Aid	LGM	NIEHS

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: We are currently using the bacteriophage Mud(Aplac) 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 Mud(Aplac) phage into the chromosome may not be entirely random.

There are, however, a number of distinct advantages to using this phage to select for mutants. This Mud(Aplac) phage carries a sufficient amount of the Mu genome to allow insertion into the E. coli chromosome and an ampicillin resistance marker. This Mu phage was constructed by Casadaban and Cohen (1979, Proc. Natl. Acad. Sci., 76:4530). It has four features which are important. The first feature is that Mu apparently inserts at random and ampicillin resistance marker allows the selection for random insertions into the chromosome. The second feature is that the phage also carries the structural genes of the lactose operon, however, it lacks a promoter and is therefore unable to synthesize beta-galactosidase (β-gal). The third feature is that there are no mRNA stop sequences between the lactose end of the phage and the lactose operon. Thus when Mud(Aplac) has inserted into a gene in the proper orientation β-gal can be synthesized using the promoter of the gene into which the insertion has occured. Messenger synthesis begins at the external promoter and proceeds into Mu through the lactose operon. This dependence upon the external promoter for β-gal synthesis results in the additional property that β-gal synthesis will be under the control of the external regulatory sequence and will be expressed only when this external promoter operator region is derepressed. The fourth feature is the effect that Mu insertion has on the gene into which insertion has occurred. Such insertions can lie either within the protein coding region of the gene, the leader sequence or the tail (the region between the end of the protein coding sequence and the end of the messenger RNA). If the insertion is in any region except possibly the tail, then the product of the gene harboring the Mu insertion will be destroyed and a mutant phenotype will result. Thus the Mu phage also acts as a mutagen.

Taking advantage of these four properties of the Mud(Aplac) phage we have selected a number of insertions which induce β-gal activity in response to treatment with low levels of alkylating agents.

We are currently using the ß-gal assay, to determine which agents induce these gene fusions, what the kinetics of ß-gal induction are, and to isolate new regulatory mutants by selecting for strains which allow constitutive expression of the Mud(Aplac) borne ß-gal gene. Efforts are also underway to determine the loci of the Mu insertions, how many different loci are represented and what mutant phenotypes can be identified.

The mutants thus generated will allow the identification of the 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60132-03 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction of recF143 and recA441 Mutations in DNA Repair and Mutagenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael R. Volkert Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(If any)*

Department of Molecular Biology
Univ. of Calif.
Berkeley, CA

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 mutations, 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.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael R. Volkert	Senior Staff Fellow	LGM	NIEHS
Margaret A. Hartke	Biological Aid	LGM	NIEHS

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60135-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Instability in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John W. Drake Head, Mutagenesis Sec. LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 0.1% 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 has been found among spontaneous and gamma-induced rII mutants, and subjected to genetical analysis to determine their nature. Virtually all of these mutants turn out to arise at highly redundant DNA sequences composed of ...AAAAAA..., and probably to consist of the addition of one A to the series.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

John W. Drake Head, Mutagenesis Section LGM NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Forward r mutants are screened in T4 stocks untreated or treated with about 500 KR of gamma irradiation. The unstable mutants are identified by their high reversion rates. They are 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%. With only rare exceptions, these mutations map at the three classical rII mutational hot spots, now known to contain runs of six A:T base pairs.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60136-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultraviolet Mutagenesis in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John W. Drake Head, Mutagenesis Sec. LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

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Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 experiments indicate that most of the claimed reversion is a reactivation artefact. In additional tests to recover a mutation in a putative new T4 DNA repair gene, dubbed uvsZ, both the mutation of interest plus a novel T4 strain exhibiting greater than wild-type UV resistance have been recovered. Both mutations will be subjected to genetic and phenotypic analysis.

Principal Investigator and All Other Personnel Engaged on the Project:

John W. Drake Head, Mutagenesis Section LGM NIEHS

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. Mutations affecting T4 ultraviolet sensitivity are recovered from multiply mutant stocks by backcrosses to the wild type, and are characterized by their survival in standard UV irradiation tests.

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. In addition to (expected) UV-sensitive mutants, a variant has been recovered that displays survival greater than that of the wild type.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60137-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Induced Reactivation of UV Damaged øX174 Bacteriophage

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael R. Volkert Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 polymerae 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.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael R. Volkert Senior Staff Fellow LGM NIEHS

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 /X174. 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 analogous 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 as compared with 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60138-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Suppression of recF Mutations

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael R. Volkert Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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, sbcB and recF. In this genetic background a large proportion of DNA repair and all genetic recombination 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 allow recombination and repair to proceed in the absence of either wild type recBC or recF activities.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael R. Volkert	Senior Staff Fellow	LGM	NIEHS
Margaret A. Hartke	Biological Aid	LGM	NIEHS

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: srfA mutations are suppressors of recF that map in recA. The suppression of recF caused by srfA appears to be different from that caused by recA441 in that protease activity is not expressed in the absence of DNA damage and the suppression is not excision dependent. The srfA suppression restores the RecF pathway of recombination to recF mutants in the recB recC sbcB genetic background. Our working hypothesis is that the RecF pathway of recombination in wild type strains results from a recF mediated modulation of recA activity and that srfA is a mutation that has caused a similar modulation of recA activity.

The recent demonstration that the RecF pathway of recombination is inducible and controlled by lexA (ie. an SOS gene dependent pathway of recombination) suggests that this pathway of recombination may serve a repair function in wild type cells and that sbc mutations allow the RecF pathway to function in genetic recombination as well.

According to this hypothesis the recF role in the regulation of the SOS response is to activate the recA protease activity and the RecF pathway of recombination results from a recF mediated modulation of recA activity. The recA441 mutation allows one of these changes, protease activation, to occur in the absence of recF and srfA results in the other change, activating the RecF pathway of recombination. In wild type cells recF causes both of these changes to occur in response to DNA damage activating simultaneously both the recA protease activity and the recA activity which allows the RecF pathway of recombination to function in DNA repair. sbc mutations allow this repair recombination to function in normal genetic recombination.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60141-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of a Rapid Procedure to Obtain DNA Sequence from T4 Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Frances W. Coleman Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The bacteriophage T4 is a powerful tool in the analysis of basic mechanisms of mutagenesis. The genetic information of rII region has been utilized extensively, resulting in the proposition of specific mutagenic pathways. Direct confirmation of predictions made by relying on T4 genetic data is now possible using DNA cloning and sequencing. Since analysis of large numbers of mutants is involved, a technique for sequencing specific regions of T4 DNA without prior cloning of each individual mutant fragment is being developed.

Principal Investigator and All Other Personnel Engaged on the Project:

Frances W. Coleman Staff Fellow LGM NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Restriction enzyme analysis. DNA-DNA hybridization. ^{32}P-labeling of specific DNAs by nick-translation. Purification of *in vivo* ^{3}H-labeled T4 DNA. Sanger dideoxy DNA sequencing. Agarose and acrylamide gel electrophoresis. Southern transfer of DNA fragments to nitrocellulose.

MAJOR FINDINGS AND PROPOSED COURSE: Although most restriction enzymes are unable to cleave T4 DNA as it contains glucosyl-hydroxymethyl cytosine, T4 DNA has been successfully digested by restriction enzyme Aha III, and Aha III sites in the rII region of T4 have been mapped.

For purposes of developing a method to purify mutant T4 DNA fragments for sequencing, ^{3}H-DNA has been prepared from a T4 deletion mutant lacking the rII region and cloned rII region DNA has been ^{32}P-labeled by nick-translation. The DNAs will be digested to completion with the restriction endonuclease Taq I, denatured, and annealed to nitrocellulose filters bearing a recombinant M13 phage containing a cloned Taq I fragment from T4 rII region. The procedure will be refined to maximize ^{32}P-DNA and minimize ^{3}H-DNA retention on the filter. The DNA fragment obtained by this enrichment technique will be melted off the filters, a specific DNA fragment will be annealed as a primer and the DNA will be sequenced using the Sanger dideoxy chain termination procedure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Much of the knowledge of basic mechanisms of mutagenesis has been inferred from the genetic system of phage T4. Sequencing of T4 mutants would allow direct confirmation of proposed pathways of mutagenesis by environmental mutagens. The techniques described here would considerably expedite sequencing of specific mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60142-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of DNA Sequence on Deletion and Frameshift Events

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Barry W. Glickman Expert LGM NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The extent to which primary DNA sequence effects the degree and specificity of mutagenesis is being determined in a forward/reverse mutational assay employing genetically engineered derivatives of M13mp2. Recent models for the formation of frameshift and deletion formation propose that besides repeated sequences, palindromic DNA sequences may also provide structural intermediates for their formation. The development of recombinant DNA technology allows this possibility to be tested. Various DNA targets are being constructed which differ in one or more of the following ways: a) base composition, e.g., A:T or G:C richness, b) length of a run of same base, c) number of repeats of a common sequence, d) palindromic or quasipalindromic sequences, putatively able to form secondary structures. Mutagenesis of these targets can then be used to monitor DNA sequence alteration and their characterization should yield information on the mechanism of their formation. These DNA constructs will also be analyzed in in vitro studies using purified replication and repair proteins. In addition, the development of plasmid constructs which mutate by a known mechanism will allow an investigation of the role of host DNA metabolism in driving these events.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Barry W. Glickman	Expert	LGM	NIEHS
Ronnie L. Dunn	Biologist	LGM	NIEHS
Thomas A. Kunkel	Senior Staff Fellow	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The development of a series of M13mplac plasmids has provided us with a gene (lacZ, coding for -galactosidase) whose expression in E. coli can be easily measured (when the gene product is present the plaques are blue on X-gal) and which contain a diversity of DNA restriction enzyme cut sites into which specific DNA sequences can be inserted. The insertion of such DNA sequences, when it alters the reading frame of the gene (i.e. when the insert is not a multiple of three) results in a disruption of the gene and as a result the plaques formed are white rather than blue on X-gal containing medium. The reversion of these constructs can then be monitored by screening for blue plaques of sectors on the same medium. Forward mutants can be detected as "white" plaques. Thus mutants can then be analyzed by DNA sequencing to characterize the changes at the DNA sequence level.

Initial experiments are centered on two different types of constructs. In one case repeated sequences (EcoRI linkers) have been inserted into the EcoRI site of lacZ in M13mp2 to produce a series of constructs carrying different numbers of the repeated DNA sequence and in the second case, a 66-base pair perfect palindrome is being inserted into that site.

MAJOR FINDINGS AND PROPOSED COURSE: The spontaneous frequency and spectrum of forward M13mp2 mutants, the parent strain for these studies, has been established, including the sequence analysis of 87 mutant phage. The first constructions, additions of multiple copies of the EcoRI linker sequence GGAATTCC, have been performed and mutation frequencies from these have been determined. The frequencies are proportional to the number of copies of the repeat, which in the mutants are lost in discreet units of eight or multiples of eight nucleotides. From one such mutant we have been able to construct a DNA sequence which may allow us to assess the relative frequency of mutagenesis due to the loss of direct repeats versus a perfect palindrome. Several other constructions are planned using runs of the same nucleotide or direct repeats of a common sequence to examine the properties of slippage of DNA sequences for their effects on mutagenesis. Such constructions will eventually be used for several diverse in vivo and in vitro mutagenicity studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Deletions, frameshifts and insertions have been accredited responsibility for a large percentage of spontaneous mutation in E. coli. It has been known for some time that the actual DNA sequence has a major influence on both the frequency and specificity of these events, both spontaneous and induced by DNA damaging agents. Classically these mutations have been thought to be the result of

structural misalignments permitted by repeated DNA sequences which have somehow been captured by subsequent DNA metabolism. Recently, we have suggested that palindromic DNA sequences may also provide a DNA context for these misalignments. The goal of this proposal is to define the relative importance of properties of specific DNA sequences on mutagenesis. In addition we wish to better understand the role of host DNA metabolism in promoting these events. At present very little is known concerning the mechanics of the events and this approach provides a method to gain insights into the molecular details of these major sources of mutation. Such knowledge should aid our understanding of mutagenesis in general, and should permit a more accurate assessment of sequence specific affects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60143-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mutagenesis in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Barry W. Glickman & Richard A. Zakour Expert/Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Little is known about the nature of mutation at the DNA sequence level in mammalian cells. The emerging new biology utilizing recombinant DNA technology makes it possible to investigate mutagenesis at the DNA level in the mammalian genome. This project is directed at that goal and involves: (1) The construction of appropriate shuttle vectors; (2) The development of genetic mapping systems for the pre-localization of mutations; and (3) The development of recovery and sequencing technology for the characterization of mutants.

Principal Investigator and All Other Personnel Engaged on the Project:

Barry W. Glickman	Expert	LGM	NIEHS
Richard A. Zakour	Staff Fellow	LGM	NIEHS
Roel Schaaper	Visiting Fellow	LGM	NIEHS
Donald Halderman	Biologist	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this project is to develop a system for the repeated determination of the exact nature of mutational changes at the level of DNA sequence in mammalian cells. This will require the recovery and cloning of a genetic target that has been mutated in the cell. The use of E. coli plasmid vectors will allow the DNA sequencing of each mutant. In order to do this the target gene must be able to be expressed in both the mammalian and bacterial cells. The shuttle system entails the insertion of a specific gene into a defined DNA context into a mammalian chromosome, the recovery of these genes from the mamalian cell, and their recloning into E. coli for molecular characterization.

1. Plasmid construction. A recombinant DNA plasmid will be constructed containing two or more markers that are expressed and thus selectable in mammalian cells and a bacterial marker to facilitate plasmid recovery in E. coli. Deletion mapping requires that the target genes also be expressed in E. coli. This will provide preliminary localization to facilitate DNA sequencing.

2. Gene transfer. The introduction of the constructed plasmid will be done by DMGT into cells in which the transfered genes can be selected. Growth conditions can be chosen to select for cells that have taken up the DNA and express the desired gene product. The selective pressure will be maintained over several generations to assure the stable integration of the plasmid DNA into the chromosome of the mammalian cell. Our goal will be to isolate stable transformants containing a single copy of the plasmid.

3. Mutagenesis. Mutants are to be selected after induction by various treatments (e.g. UV light, chemical carcinogens) or arising spontaneously. The use of a multi-gene system is desirable because it allows selective pressure to be maintained on one of the plasmid genes while selecting for mutants of the other. The genetic system is designed so that reversion can also be monitored.

4. Recloning. The mutated gene must be efficiently recloned from the mammalian cell. Several approaches shall be tested to determine the most efficient. The first is to transform E. coli using total cellular DNA from the mutated cells. Alternatively, enrichment by biochemical and biophysical means shall be done. The recloned plasmid DNA once grown in the E. coli will be used for sequence analysis.

5. Sequencing. Characterized deletion mutants will be used to first localize the site of the induced mutations by genetic means in E. coli. Then, the DNA sequence of the mutant gene will be determined by established techniques.

MAJOR FINDINGS AND PROPOSED COURSE: To date, progress has been made in the first two areas described above. This will be described.

(1) Plasmid construction. A recombinant pBR322-derivative plasmid (pTK/GK) has been constructed that carries the thymidine kinase (TK) gene from Herpes Simplex Virus and the galactokinase gene (GK) from E. coli. The galactokinase gene is under control of the early SV40 promoter and, the plasmid from which it was derived also provides an SV-40 splice region and a polyadenylation sequence. The thymidine kinase gene is under control of the Tet-promoter of pBR322. This construction allows the expression of both genes in mammalian cells and in E. coli. The plasmid also contains the ampicillin resistance gene (AMP^r) from pBR322 which allows for selection in E. coli. The plasmid also contains several unique restriction enzyme cut sites in non-coding segments of DNA. The latter feature is important as it should facilitate efficient recovery of the plasmid from the transformed mammalian cell.

(2) Gene transfer. The p(TK/GK) plasmid has been used for all DNA mediated transformation experiments to date. The initial attempts at DMGT were done using mouse lymphoma line 191.3 which are TK^- GK^- cells that grow in suspension culture (in collaboration with Dr. D. Clive of Burroughs Wellcome Company). This approach was discontinued as the transformation efficiency was lower than the spontaneous back mutation frequency for the TK locus ($<10^{-6}$). Using the TK^- mouse L cell line B6 high transformation effienciencies ($>10^{-3}$) were obtained for the TK^+ phenotype. Cell lines obtained from individual transformed clones have been isolated from these experiments and are currently being grown for further characterization (both phenotypic and molecular). We have also isolated spontaneous GK^- mutants from mouse L cell line B6 for use in future experiments with p(TK/GK) plasmid. Other plasmids that we have available containing selectable markers include the gene for the E. coli enzyme xanthine-guanine phosphoribosyl transferase (XGPRT) which can substitute for the X-chromosome linked gene for the mammalian enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) in cells, and the gene for resistance to the antibiotic neomycin (G418). The latter gene has the advantage that it can be used as a dominant selectable marker for any mammalian cell line. Each of the different plasmids will be tested alone and combined in co-transformation experiments to obtain transformed cell lines containing stable intergrated functional plasmid DNA for use in our mutagenesis studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project provides a molecular approach to mutagenesis in mammalian cells. The availability of spontaneous and induced spectra from mammalian cells will provide important clues to the mechanism of mutation in mammals (Are mutations targeted by lesions? Do agents show the same specificity in mammal as E. coli? Are mobile genetic elements of importance?) Not only will a better understanding of the mutational process be likely but improved ability to estimate risks posed by improved environmental agents will also result.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60144-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Depurination-induced Mutagenesis in a Forward Mutational Assay

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Thomas A. Kunkel Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Depurination, the loss of a purine base from DNA, has been shown to be highly mutagenic in a forward mutational assay using M13lac DNA. DNA sequence analysis of 213 mutants indicates that this DNA damage primarily results in base substitution mutations, while only a small increase in frameshift mutagenesis is observed. The mutagenicity is predominantly observed in the presence of error prone DNA synthesis and exhibits a strong specificity for insertion of dAMP opposite the apurinic site, resulting primarily in transversions. These data in a forward mutational system provide insight into the mechanisms used by a cell to replicate DNA containing non-coding lesions.

Principal Investigator and All Other Personnel Engaged on the Project:

Thomas A. Kunkel	Senior Staff Fellow	LGM	NIEHS
Joyce Liu	Biologist	LGM	NIEHS
Jennifer Motto	Biologist	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mutagenicity is examined using the single-stranded bacteriophage M13mp2, which contains the α-peptide coding region of the lacZ gene of E. coli. Mutants obtained from a transfection assay using normal or depurinated DNA, are scored as having a light blue or colorless phenotype compared to wild type blue color, when plated on the appropriate indicator plates. The exact nature of the mutational events is then determined by chain terminator DNA sequence analysis.

MAJOR FINDINGS AND PROPOSED COURSE: The mutagenic consequences of damage to DNA produced by low pH and high temperature (primarily depurination)have been determined in a forward mutational system. Transfection of depurinated single strand M13mp2 DNA into competent cells results in a 15-fold increase in the frequence of mutant (light blue or colorless) plaques compared to a non-depurinated DNA control. Mutagenicity is proportional to the number of lethal sites introduced into the DNA and is largely dependent on a functional error prone repair system in the competent E. coli cells. Approximately 90% of the damage-dependent increase in mutagenicity is abolished by apurinic endonuclease or by alkali-treatment of the damaged DNA prior to transfection. Based on these observations and the rate constants for formation of the various types of heat/acid produced lesions in DNA, it is concluded that the majority of the induced-mutagenesis results from the presence of abasic sites in the DNA. DNA sequence analysis of 87 spontaneous and 124 induced mutants indicates that three types of mutational events are increased: base substitutions (31-fold), double mutations (>33-fold) and mutations arising from recombination between the M13mp2 DNA and the complementary lac information on the F' in the host cell (16-fold). There is only a slight increase in the frequency of frameshift mutations (3.8 fold) and essentially no increase in large deletion mutations. Approximately 80% of the base substitution mutations occur at purine positions in the viral strand, consistent with depurination as the predominant premutagenic lesion. The 2:1 ratio of G:A sites mutated is consistent with the preference for depurination of G over A. Transversions are observed for 57 of 79 (72%) induced base substitutions, with a strong preference for insertion of A opposite the putative apurinic site.

These studies will be continued to address several important issues including the relevance of depurination-induced mutagenesis in double stranded DNA and in higher organisms and the reason(s) underlying the highly unexpected preference for insertion of dAMP opposite this non-coding lesion.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Depurination has been estimated to be a frequent cellular event, with 10,000 purine bases lost in a single mammalian cell per generation. The rate constant

for depurination increases dramatically with certain types of DNA base modifications produced by several known mutagens and carcinogens. This potentially large loss of genetic information, when unrepaired, may present a significant mutagenic challenge to a cell, and may be responsible for many spontaneous and induced mutational events. These experiments are designed to increase our understanding of how an organism copes with such DNA damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60145-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Mutagenesis with Purified DNA Replication and Repair Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Thomas A. Kunkel Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

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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 *(Use standard unreduced type. Do not exceed the space provided.)*

The fidelity of purified DNA polymerases are being assessed in in vitro DNA synthesis assays involving biologically active DNA templates. Both a forward mutational assay, using M13mp2 phage and capable of detecting a wide spectrum of base substitution and frameshift errors, and a reversion assay, using either ϕX174 DNA or M13mp2 DNA and capable of detecting relatively specific errors, are being employed. Highly purified DNA polymerases from well characterized procaryotic systems and less well defined eucaryotic organisms all produce base substitution errors in vitro at detectable levels. The exact nature of these errors at the level of DNA sequence is currently underway, as is the role of these enzymes in producing frameshift errors. These studies are intended to provide detailed information on the accuracy of the DNA polymerases themselves, as well as information of the parameters of protein nucleic acid interactions which are important in determining this accuracy.

Principal Investigator and All Other Personnel Engaged on the Project:

Thomas A. Kunkel	Senior Staff Fellow	LGM	NIEHS
Joyce Liu	Biologist	LGM	NIEHS
Jennifer Motto	Biologist	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Highly purified DNA polymeraes are used to synthesize a DNA strand in vitro, using as a template the single stranded cricular DNA from either M13mp2 or øX174 phage. The DNA sequence which is the target for mutagenesis can be chosen to analyze either base substitution errors, frameshift errors, or both. The newly synthesized compelentary strand, containing the mutation to be analyzed, is expressed by transfection of the product of the in vitro reaction by transfection into competent E. coli cells (either $CaCl_2$ cells or spheroplasts). Mutants are selected on the basis of plaque color (M13mp2) or ability to grow on suppressor minus bacteria (øX174), and are analyzed by DNA sequencing.

MAJOR FINDINGS AND PROPOSED COURSE: The M13mp2 forward mutational assay has been established and shown to be applicable to in vitro analysis of DNA polymerase fidelity. The following DNA polymerases produce detectable forward mutagenesis in vitro: E. coli DNA polymerae I, AMV reverse transcriptase, calf thymus DNA polymerase-α, rat hepatoma DNA polymerase α and calf liver DNA polymerase-α. An analysis of the DNA sequence of mutants produced by these enzymes is in progress, with approximately six mutants analyzed for each enzyme. A determination of the exact error rates, which requires a number of controls, is in progress. The studies will be expanded to reversion assays for specific frameshifts and base substitutions and to the use of specific new derivatives of M13mp2 containing variations in the primary DNA sequence target. More importantly, in the long term, the analyses will be expanded to the use of more complex in vitro DNA synthesis systems, since it is well known that DNA polymerases work in concert with other proteins in vivo.

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 for the crucial enzymes involved in the syntehsis of the genetic information, the DNA polymerases themselves. Such information is necessary for 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60146-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutagenic Consequences of Site Specific Introduction of a Defined Lesion into DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Thomas A. Kunkel Senior Staff Fellow LGM NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

These experiments are designed to determine the mutagenic consequences of the introduction of a single DNA lesion into a known position of a DNA molecule. The system used is the highly defined M13lac forward mutation assay which allows detection and exact characterization of all classes of mutations both at and some distance away from the actual site of DNA damage. The first application of this approach, currently underway, is to determine the specificity of the mutagenesis resulting from the introduction of a single apurinic site into several individual positions in the DNA sequence which codes for the α-complementing activity in the lacZ gene carried in M13mp2 phage. Mutants, produced under conditions of normal or altered DNA replication and/or DNA repair capacity, will be scored and defined by DNA sequence analysis. This approach is designed to overcome limitations to classical approaches which leave uncertainty as to whether the mutations recovered actually occur at the site of the lesion. Furthermore, an examination of these lesions at several locations also permit a determination of neighbouring base pair effects on the mutational specificity of such lesions. The results should provide substantial information on the specific mutagenic consequences of defined DNA lesions in biological systems.

Principal Investigator and All Other Personnel Engaged on the Project:

Thomas A. Kunkel	Senior Staff Fellow	LGM	NIEHS
Roel Schaaper	Visiting Fellow	LGM	NIEHS
Barry W. Glickman	Expert	LGM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Oligonucleotides of known sequence, containing the desired single DNA lesion, are synthesized by the soluble phosphotriester method. The oligonucleotide is chosen to be complementary to a specific site in M13*lac* single-stranded DNA, and is used as a primer for synthesis of a covalently closed circular complementary strand, using *E. coli* DNA polymerase I (large fragment) and T4 DNA ligase. This complementary strand, containing the lesion at a position known to produce detectable mutations, is then purified and transfected into $CaCl_2$ cells having various DNA replication and repair phenotypes. Mutants are scored as having altered ability to produce functional β-galactosidase activity, and are sequenced by the chain terminator method. Illustrated below is the sequence of the first primer fragment used in this study. Sites where mutation has been shown to result in an altered phenotype are marked with an asteric.

```
                                  * * * *   * *   * *
(- strand)      5'- T T T T C C C A G T C A C G -3'
```

MAJOR FINDINGS AND PROPOSED COURSE: The M13*lac* forward mutation assay has been developed in detail, and the ability of apurinic sites to cause mutation in the system has been determined. Two oligonucleotides have been synthesized as complements to a specific DNA sequence in which all possible base substitutions as well as frameshift, deletion and additional mutations can be scored. One of these oligonucleotides contains a uracil, confirmed by DNA sequence analysis, which will be removed by uracil glycosylase to give the apurinic site. The details for synthesis and purification of the covalently closed complementary strand are determined, and the mutagenic consequences of the single apurinic are now being determined in cells expressing error prone DNA synthesis (i.e. SOS-repair). These studies will be expanded to include several different positions for the apurinic site as well as different replication and repair backgrounds. Ultimatly the system will be used for other defined DNA lesions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the major limitations of previous studies on the effects of DNA damage in biological system is that all DNA damaging agents produce a spectrum of different DNA lesions. It is therefore difficult to assign a biological endpoint to a specific lesion. These studies are intended to overcome this limitation and provide detailed information on the exact mutagenic consequences of a single type of damage to DNA. Such information is critical to our understanding of the risk posed by various DNA damaging agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61005-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and Function of RNA Polymerase II in Drosophila melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert A. Voelker Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

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

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was initiated to genetically analyze the biosynthesis and function of the components of the 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 now been cloned as recombinant DNA molecules and was found to encode the 215,000 dalton subunit. The genetic control of the biosynthesis of that subunit is being analyzed at the molecular level by analyzing a number of revertants of the P-element induced mutant that was used to clone the DNA sequences of the region.

Principal Investigator and All Other Personnel Engaged on the Project:

Robert A. Voelker	Research Geneticist	LG	NIEHS
Lillie L. Searles	Staff Fellow	LG	NIEHS
Shu-Mei S. Huang	Biological Laboratory Technician	LG	NIEHS
G. Bruce Wisely	Biological Laboratory Technician	LG	NIEHS

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 and 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 that confers α-amanitin resistance to RNA polymerase II and encodes the 215,000 dalton subunit has been identified. The locus is lethal-mutable. Different alleles at the locus affect male fertility and act as enhancers of alleles at the other loci. To date, we have recovered at least 8 temperature-sensitive mutants. 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 molecularly cloned by identifying and recovering mutants at the locus that were caused by hybrid-dysgenesis-induced insertion of the P-element transposon. One of these P-element-induced mutants has been found to be genetically very unstable in dysgenic flies. It reverts with frequencies of 2.5 to 6.5 percent by excision of the P-element, with many of the revertants resulting from imprecise excision. The consequences of these imprecise excisions on function are being analyzed.

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.

PUBLICATIONS

Searles, L.L., Jokerst, R.S., Bingham, P.M., Voelker, R.A., and Greenleaf, A.L.: Molecular cloning of sequences from a Drosophila RNA polymerase II locus by P element transposon tagging. Cell 31: 585-592, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61011-04 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organization and Regulation of Gene Function in D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

B. H. Judd Chief LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study combines the genetical, cytological and molecular characterization of the white locus of Drosophila melanogaster. It involves comparisons of the normal gene to mutant forms that perturb the regulation of the locus. The mutations currently under investigation result from the insertion/deletion of transposable elements near the 5' end of the gene. The molecular nature of the changes are being determined to obtain information about the mechanisms of regulation for the locus and to learn more about the nature of the mutational events that affect regulation.

Principal Investigator and All Other Personnel Engaged on the Project:

Burke H. Judd	Chief	LG	NIEHS
Margaret W. Shen	Biologist	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The analysis is by genetic, cytological and molecular characterization of specific genes in D. melanogaster. Genetic analysis involves recombination, complementation tests and induction of mutations. Cytological preparations provide information about chromosome aberrations and the in situ hybridizations of labeled DNA probes give information that ties together the genetical and molecular information concerning gene structure. Molecular techniques include restriction enzyme digestion of DNA, Southern blot analysis, nick translation, and nucleotide sequencing.

MAJOR FINDINGS AND PROPOSED COURSE: This analysis of the white locus of Drosophila melanogaster addresses questions about the organization, function and regulation of eukaryotic genes. We want to understand the mechanism through which genes respond in developmentally regulated patterns to produce the multiple cell and tissue types of complex organisms. The white locus provides a model system through its many mutant forms and the interactions it has with other loci. A study of mutant alleles that perturb the regulation of the locus is providing information about the nature of the regulatory elements of the gene and also about the nature and mechanisms of gene mutation. The mutation w^{zm} produces a mosaic pattern of eye pigmentation. This mutation is associated with the insertion of a transposable sequence of DNA near the 5' end of the transcribed portion of the gene and it exhibits a moderate instability. Mutant derivatives of w^{zm} show striking modifications of gene regulation, with some exhibiting mosaic patterns that are clonal and autonomous (w^{zk}), others completely lack any pigmentation (w^{z}), while the most unusual group shows a nonclonal, nonautonomous mosaic of pigment deposition (w^{zh}). It has been determined that the w^{z} mutation is the result of a second insertion of nonwhite locus DNA about 3.5kb from the site of the w^{zm} insertion. The nonclonal, nonautonomous type such as w^{zh} has lost most of both the insertion sequences in w^{z}; in addition the entire white locus as well as some adjacent genes have been transposed to the left arm of chromosome 3. We plan a detailed analysis of the gene regions into which the transposons are inserted and a comparison of different mutant forms to determine how they affect the normal regulation of the locus. Of particular interest is a comparison of the mutants showing the clonal expression of the gene compared to those giving nonclonal action. In both there seems to be improper gene activation but this happens in some cells only. In the clonal form those cells that have proper activation, pass the activated state on through several mitotic divisions of daughter cells. In the nonclonal mutants this mitotic memory is not functioning or possibly the locus is not activated in the proper tissues and thus non-autonomy results.

Another aspect of white locus structure being investigated is the molecular basis for intralocus asymmetrical exchanges that result in small duplication and deficiency recombinant chromosomes.. Preliminary data implicate a

repeated transposable DNA sequence, B104, that is associated with the w^{bf} mutation. This element apparently is located at a different place in or near the white locus of the w^{a} chromosome. Pairing and exchange between the B104 sequences in the two chromosomes creates the duplication and deficiency products. These products are useful in characterizing regional differentiation within the locus and for studying the details of the mechanisms involved in crossing over.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will provide basic information about gene structure and function that will help in understanding eukaryotic genes as targets for environmental mutagens and that will lead to determination of the mechanisms by which genes are developmentally regulated in multicellular organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61018-03 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Sequence Variation in the Alcohol Dehydrogenase Gene Region of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Charles H. Langley Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

Dr. C. Laurie-Ahlberg, Associate Professor of Genetics
North Carolina State University, Raleigh, North Carolina

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Variation in the restriction map in the Adh region (alcohol dehydrogenase locus) of chromosome II of Drosophila melanogaster from natural populations was examined. Insertion/deletion differences were common. All insertions of over 200 nucleotides share sequence homology with known transposable elements. The distribution within Drosophila melanogaster and among related species suggest that such variants are deleterious mutants. Preliminary comparisons of gene activity among inserted and noninserted sequences supports this view.

Principal Investigator and All Other Personnel Engaged on the Project:

Charles H. Langley	Research Geneticist	LG	NIEHS
Charles F. Aquadro	Staff Fellow	LG	NIEHS
Susan F. Deese	Biological Laboratory Technician	LG	NIEHS
William F. Quattlebaum	Biological Laboratory Technician	LG	NIEHS
George R. Carmody	Guest Worker	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Fifty genetically isolated second chromosomes of Drosophila melanogaster were reared and nuclear DNA isolated. Restriction maps were constructed for the Adh region and compared for evidence of nucleotide substitutions and insertion/deletion variation. Insertions and deletions were cloned and are being further analyzed by detailed restriction mapping and DNA sequencing. Homology of DNA associated with insertions/deletions to known transposable elements was also examined. Gene activity (enzymatic activity and messenger RNA levels) is being measured to assess the effects of sequence alterations.

MAJOR FINDINGS AND PROPOSED COURSE: Six restriction site polymorphisms and a minimum of 15 unique insertions/deletions within the 12 kilobase region surrounding Adh were found in the 50 randomly chosen second chromosomes from natural populations of Drosophila melanogaster. Only three small (<100 nucleotides) insertions/deletions occurred within the Adh transcriptional unit (within a intron). An apparent "hot spot" for insertion/deletion occurs 3' to the Adh gene. Small insertion/deletion events (less than 200 nucleotides) appear to involve unique sequences. In contrast, all seven sizes of insertions (ranging from 0.4 - 9.0 kilobases) have homology to known transposable elements. Six of these seven sizes of insertions are unique to a single chromosome, while one occurred in five different chromosomes. Examination of the restriction maps of those chromosomes, however, suggest these five represent as many as five independent insertion events. Thus, in contrast to small unique sequence insertions/deletions and restriction site polymorphisms which often reach high frequency, transposable element insertions are individually very rare even though they are a common class of variants. These results and the previous observation of little insertion/deletion evolution among D. melanogaster and its closest relatives suggest these insertions and deletions produce small deleterious effects (transposable elements more so than the smaller insertions/deletions). Preliminary analyses of gene activity appear to support this conclusion. Within the next year, we will complete the sequence analyses of the sites of insertion/deletion and the analysis of the effect of these lesions on gene expression.

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 (particularly transposable elements) in and outside transcriptional units is unclear but

the question is approachable. These results contribute to the understanding of domains responsible for proper gene expression and define genetic lesions that can upset that regulation.

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 79: 5631-5635, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61019-03 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Collaborative Protein Sequencing

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Steven S.-L. Li Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

Department of Pharmacology, University of North Carolina, Chapel Hill, NC
Department of Diagnostic Immunology Research and Biochemistry
Roswell Park Memorial Institute, Buffalo, New York

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Amino acid sequence of about 95% of the 186 residues from human dihydrofolate reductase has been determined.

A new human prostatic acid phosphatase isozyme has been purified and characterized.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Steven S.-L. Li	Research Geneticist	LG	NIEHS
Farida S. Sharief	Biologist	LG	NIEHS

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: Amino acid sequence of human dihydrofolate reductase has been determined, and the primary structure information is also helpful to understand the structure-function relationship of dihydrofolate reductase.

A new human prostatic acid phosphatase isozyme has been purified and characterized.

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 of these molecules. 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., Domin, B.A., Li, S.S.-L., and Cheng, Y.-C.: Amino acid sequence studies of dihydrofolate reductase from a human methotrexate resistance cell line - structural and kinetic comparison with mouse L1210 enzyme. Eur. J. Biochem., 132: 351-359, 1983.

Lin, M.F., Lee, C.-L., Li, S.S.-L., and Chu, T.M.: Purification and characterization of a new human prostatic acid phosphatase isozyme. Biochemistry 22: 1055-1062, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61021-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Molecular Analysis of the cut Locus of D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Joseph W. Jack Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure 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 *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in knowing how cells of a single organism can differentiate to form specific tissue types. We have chosen to address one aspect of the question by learning how one gene, the cut locus of Drosophila melanogaster, is expressed differently in different tissues of the fly.

The analysis of a number of mutants of the cut locus suggests that there are at least two regions of the gene, each of which is necessary for gene activity in a limited and nonoverlapping set of tissues. Another region is possibly necessary for expression throughout the fly.

The availability of tissue specific mutants of a gene afford the opportunity to experiment to find out how the gene normally operates in tissue specific ways. We are currently studying the transcriptional activity of the cut locus and the DNA structure of normal and mutant alleles with the intention of finding out what causes the mutations and how the activity is altered.

We now know that many of the cut mutants are insertions of retrovirus-like sequences into the cut locus DNA, and we are interested in understanding the affect of these sequences on gene activity.

Principal Investigator and All Other Personnel Engaged on the Project:

Joseph W. Jack	Staff Fellow	LG	NIEHS
Willie Gibson	Research Chemist	LG	NIEHS
Angela E. Lane	Biological Laboratory Technician	LG	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: In the past year we have studied the DNA structure of a number of mutant cut alleles. We find that the leg specific mutant kf is caused by the deletion of sequences in the left most portion of the locus. Eight wing mutations all map to the right of the kf deletion and are all caused by the insertion of 5-10 kb DNA segments. DNA from three of these mutants has been cloned, and all insertion elements are repeated sequences. One is homologous to the transposable element copia, and another has been identified as the transposable element B104. Both of these elements are similar in structure and activity to mammalian retroviruses. One mutant allele that manifests both leg and wing mutant phenotypes is a deletion overlapping the kf deletion and extending into the region where most cut wing mutants have been located. These results suggest that separate regions of the gene are necessary for its expression in the legs and in the wings.

A number of cut mutants generated by an unstable chromosome have been analyzed in collaboration with Johng Lim. Two of these lesions have proven to be new insertions. However, a number of the mutations are the result of an unusual genetic event. The ct^6 allele containing the transposable element gypsy was passed to the F_2 offspring of a ct^6 bearing female without being expressed in the hemizygous F_1 males. The basis for this unusual event, an apparent consequence of the unstable nature of the parental ct^6 chromosome, is yet to be understood.

We have begun to study the transcriptional activity of cut. A 1.8 kb transcript has been detected with sequence probes from throughout the 10 kb region in which cut mutants have been located. A number of cDNA clones with homology to the same cut sequences have also been recovered.

We are continuing to clone cut locus sequences that we don't already have. We will use them and previously obtained clones to analyze the mutants and transcription of the cut locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our analysis of the cut locus is aimed at understanding how genes are regulated during the development of a higher organism and how the process of regulation can be altered by various types of mutations. The analysis of the changes in cut locus activity caused by retrovirus-like sequences will be important for understanding how such sequences affect genetic activity. This understanding will be important since retroviruses are known to alter the behavior of cellular genes, in some cases giving the altered cells the potential to form tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61022-02 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Population Genetics of Transposable Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Charles H. Langley Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

Dr. Norman Kaplan
Biometry and Risk Assessment Program

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The distribution of transposable elements within and between Drosophila species were surveyed. The frequencies of particular transposable elements at various sites indicated that no site is commonly occupied. A theoretical model of the evolution of transposable elements in Mendelian populations was put forward. Analysis of the distributions among populations and species suggests that most transposable elements are stable components of the Drosophila genome, although their location and copy numbers do vary.

Principal Investigator and All Other Personnel Engaged on the Project:

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

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The distribution of transposable elements was studied in Drosophila. The variation in location in the X chromosome elements was examined in a single population of Drosophila melanogaster. These results in conjunction with theoretical analysis suggests that these elements rarely reach a high frequency at any one site. Analyses of many families of transposable elements in several related species indicate that most transposable elements are permanent components of the genome, although average copy number and location are variable.

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.

PUBLICATIONS

Langley, C.H., Brookfield, J.F.Y., and Kaplan, N.: Transposable elements in Mendelian populations. I. A theory. Genetics 104: 457-471, 1983.

Montgomery, E.A., and Langley, C.H.: Transposable elements in Mendelian populations. II. Distribution of three copia-like elements in a natural population of Drosophila melanogaster. Genetics 104: 473-483, 1983.

Kaplan, N., and Brookfield, J.F.Y.: Transposable elements in Mendelian populations. III. Statistical results. Genetics 104: 485-495, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61023-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Drosophila Germ Cell Determination

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert E. Boswell Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is a fundamental concept in developmental biology that the fate of embryonic cells is regulated by morphogenetic determinants localized in the ooplasm. In Drosophila, heterotopic transplantation experiments have conclusively demonstrated that cytoplasmic factors localized to the posterior pole plasm of the oocyte and embryo are requisite for the formation of pole cells, the primordial germ cells. However, the molecular nature of these cytoplasmic factors, the mechanism of localization within the ooplasm, and their mode of action in development are unknown.

The genetic and developmental analysis of maternal effect mutants that affect pole cell formation in Drosophila melanogaster are intended to allow one to elucidate the mechanism of determination and how the determined state is maintained throughout development.

Principal Investigator and All Other Personnel Engaged on the Project:

Robert E. Boswell	Staff Fellow	LG	NIEHS
Steven A. Haneline	Biological Laboratory Technican	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: New alleles of tudor were obtained by X-ray mutagenesis. The pole plasm of oocytes and preblastoderm embryos was analyzed by transmission electron microscopy (TEM).

MAJOR FINDINGS AND PROPOSED COURSE: A new maternal effect grandchildless (gs) mutant, tudor, will be analyzed. New alleles of tudor will be recovered by mutagenesis. Chromosomal rearrangements and temperature-sensitive alleles will be sought. The recovery of chromosomal rearrangements will allow one to further localize the locus which has been shown to be on the right arm of the second chromosome in the polytene chromosome interval 57B-58A.

The genetic analysis of tudor and other grandchildless loci will allow one to study the cytological consequences of genetic lesions leading to grandchildless phenotype. These studies will require TEM of the pole plasm of stage 13-14 oocytes as well as preblastoderm embryos. Pole cells of blastoderm embryos will also be analyzed cytologically. Scanning electron microscopy (SEM) will be used to analyze any morphological defects in developing embryos that may be associated with the abnormal segmentation. Segmentation defects can also be studied using acetylcholinesterase whole mounts and plastic sections of whole embryos. The segment defects will also be studied using Hoyer's mounts to identify the cuticle pattern defects.

Homozygous tudor females produce embryos that lack pole cells and the adults of both sexes are sterile. Like most maternal effect mutants studied to date tudor is pleiotropic. Approximately 40% of the embryos from homozygous females exhibit abnormal embryonic segmentation. The remaining 60% produce phenotypically normal adults that are sterile due to a lack of germ cells. It has been shown that various alleles of tudor contain different amounts of polar granule material, conspicuous cytoplasmic structures classically thought to be the determinants or the resident site of the determinants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A major interest within the institute is to understand the mechanisms involved in cellular determination and differentiation. Elucidation of the molecular events in determination is considered crucial to understanding medical problems such as cancer and aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61024-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Molecular Analysis of Suppressor-of-Sable Function in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert A. Voelker Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was undertaken to determine the mechanism whereby mutants at the suppressor-of-sable [su(s)] locus are able to suppress specific mutants at other loci in Drosophila melanogaster. Evidence from another Drosophila suppressor system suggests that the suppressible alleles may all be mutants caused by the insertion of a specific mobile genetic element. Our approach to answering this question will involve the molecular cloning of both the su(s) locus and the vermilion (v) locus, several alleles of which are suppressible. We will determine the product of su(s) and how it interacts at the molecular level with the suppressible vermilion alleles to effect suppression.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Robert A. Voelker	Research Geneticist	LG	NIEHS
Lillie L. Searles	Staff Fellow	LG	NIEHS
Dau-Yin Chang	Visiting Fellow	LG	NIEHS
Shu-Mei S. Huang	Biological Laboratory Technician	LG	NIEHS
G. Bruce Wisely	Biological Laboratory Technician	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The methods employed will combine an intensive genetic analysis with the molecular cloning of the gene and determination of its product. The genetic analysis will consist of lethal saturation mapping of the region. The lethals will be ordered by deficiency mapping and, if possible, correlated with the molecular structure by use of structural rearrangement-associated lethals or mobile element-induced lethals. The molecular analysis will include restriction mapping and identification of sequences which encode RNAs. If the transcript of su(s) is a poly-A^+ RNA, we will attempt to identify the protein product and determine its function.

MAJOR FINDINGS AND PROPOSED COURSE: By use of the P-element transposon tagging technique, DNA sequences of the su(s) region have been cloned. Using cloned sequences to probe DNAs from extant su(s) alleles we have determined that five spontaneous su(s) alleles possess inserts within a 3 kb region, whereas 2 X-ray induced alleles appear not to possess insertions or deletions of DNA. Preliminary analysis has identified a 2-3 kb poly-A^+ RNA which is homologous to a 7 kb probe that includes the 3 kb region in which the five inserts occur. Lethal mutants in the su(s) region have been identified and they are being tested for possible allelism to su(s). In addition a large number of deficiencies are being screened for breakpoints in the immediate su(s) region; these will be useful in ordering lethal genes and in providing molecular landmarks.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is becoming apparent that mobile genetic elements are responsible for many so-called spontaneous mutations. Since most mutations are harmful, such mutations contribute to the genetic loads of populations and in humans these mutations become causes of genetic diseases. Thus, an understanding of how these mobile genetic elements cause mutations and how an organism may evolve systems to suppress these mutations offers the prospect of minimizing the effects of such mutations or perhaps eventually eliminating them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61025-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Sequence Variation in the Dopa Decarboxylase Region of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Charles F. Aquadro Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

Dr. C. Laurie-Ahlberg, Associate Professor of Genetics
North Carolina State University, Raleigh, North Carolina

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Variation in restriction maps in a 40 kilobase region surrounding the dopa-decarboxylase structural gene (Ddc) has been examined in 50 second chromosomes from natural populations of Drosophila melanogaster. Restriction site polymorphisms indicate a level of sequence variability comparable to that seen in the Adh region. However, substantially fewer insertions and deletions are observed in the Ddc region. The significance of these patterns are being investigated through examination of Ddc gene activity and additional restriction mapping in flanking regions.

Principal Investigator and All Other Personnel Engaged on the Project:

Charles F. Aquadro	Staff Fellow	LG	NIEHS
Robert M. Jennings	Biological Aid	LG	NIEHS
Charles H. Langley	Research Geneticist	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Fifty genetically isolated second chromosomes of Drosophila melanogaster were reared and nuclear DNA isolated. Restriction maps were constructed for the Ddc region and compared for evidence of nucleotide substitutions and insertion/deletion variation. Gene activity will also be assayed.

MAJOR FINDINGS AND PROPOSED COURSE: Restriction sites for five endonucleases have been mapped in a 40 kilobase region around Ddc for 50 second chromosomes from natural populations of D. melanogaster. Nine sites are polymorphic suggesting nucleotide substitution variability of the same order of magnitude as that seen in the Adh region we have examined in these same chromosomes. However, insertions/deletions are rarer in the Ddc region than around Adh by a factor of approximately six. Five large insertions (1.5-5 kilobases) and 4 small insertions/deletions (100-300 nucleotides) occurred only once in the sample. One insertion/deletion of 250 nucleotides was very common but appears to have occurred only once and does not represent repeated events. Surprisingly, one large (2.5 kb) insert is located in the Ddc transcript, but its occurrence within an intron apparently prevents it from eliminating expression of Ddc. Significant statistical associations of restriction site variation over the entire region surrounding Ddc suggests favored combinations of alleles at loci flanking Ddc. Over the next year insertions/deletions will be cloned and analyzed to determine their origin (are they transposable elements?). Gene activity will also be examined to assess any deleterious effects of the observed lesions. Restriction map variation in regions flanking the 40 kilobase region will also be examined for an increase in insertion/deletion variation and lack of statistical associations among variants since these regions appear less "dense" genetically and should provide clues as to the significance of the patterns seen around Ddc.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The study of naturally occurring DNA sequence variation provides information on background levels of variation, information vital to the assessment of the significance of mutagen exposure. The results also contribute significantly to determining the "target" for mutagenesis and the types of variation that can and cannot be tolerated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61026-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Mitochondrial DNA Variation and Evolution

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Charles F. Aquadro Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Human mitochondrial DNA sequence comparisons have been made, resulting in the detection of high frequencies of multiple (repeated) nucleotide substitutions and insertions/deletions. Two substitution biases are apparent, one favoring transitions by a factor of 32:1 over transversions, and the other favoring a high rate of turnover of purines relative to pyrimidines on the heavy strand of mtDNA. Their occurrence in coding and non-coding regions as well as rRNA and tRNA genes suggests that these phenomena may result from biases in the mutational pathways. We have also modeled the dynamics of the substitution process in mammalian mtDNA using the information revealed by these and other comparisons. The results of these models make specific predictions about the substitution process. In addition, the models support the hypothesis that while a portion of the mtDNA molecule is relatively free to change at a very rapid rate, the majority of the molecule has very strong selective constraints on base substitutions and other types of sequence alterations.

Principal Investigator and All Other Personnel Engaged on the Project:

Charles F. Aquadro	Staff Fellow	LG	NIEHS
Norman Kaplan	Mathematician	BRAP	NIEHS
Kenneth J. Risko	Mathematician	BRAP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Published mtDNA sequences have been analyzed statistically. Mathematical models have also been constructed and their predictions studied.

MAJOR FINDINGS AND PROPOSED COURSE: We have analyzed nucleotide sequence variation in an approximately 900-base pair region of the human mitochondrial DNA molecule encompassing the heavy strand origin of replication and the D-loop. Our analysis has focused on nucleotide sequences available from seven humans. Average nucleotide diversity among the sequences is 1.7%, several-fold higher than estimates from restriction endonuclease site variation in mtDNA from these individuals and previously reported for other humans. This disparity is consistent with the rapidly evolving nature of this noncoding region. Analysis of the observed number and direction of substitutions revealed several significant biases, most notably a strand dependence of substitution type and a 32-fold bias favoring transitions over transversions. The results also revealed a significantly nonrandom distribution of nucleotide substitutions and sequence length variation. Significantly more multiple substitutions were observed than expected for these closely related sequences under the assumption of uniform rates of substitution. The bias for transitions has resulted in predominantly convergent or parallel changes among the observed multiple substitutions. There is no convincing evidence that recombination has contributed to the mtDNA sequence diversity we have observed.

The dynamics of change of mammalian mtDNA has also been modeled in an effort to isolate biologically relevant and important assumptions that influence mtDNA sequence evolution. We have drawn on published sequence data for comparison with the predictions of our model. The results confirm an extremely rapid rate of substitution and are consistent with strong underlying mutational biases and severe selective constraints on approximately one-half of the mtDNA molecule.

Additional modeling will be completed during the next year pertaining to estimated routes of substitution for mammalian mtDNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of these studies contribute to the knowledge of the extent of naturally occurring sequence variation in human mitochondrial DNA and provide a framework in which to interpret and study the nature of the substitution process in mammalian mitochondrial DNA and the nature of and susceptability to mutagenic damage due to mutagenic agents.

PUBLICATIONS

Aquadro, C.F., and Greenberg, B.D.: Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals. Genetics 103: 287-312, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61027-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transmission of Cryptic Mutations in Destabilized X Chromosomes of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Johng K. Lim Research Geneticist LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A stable Drosophila X chromosome can be destabilized by association with the unstable X chromosome (Uc) or its derivatives for only one generation. Occasionally, male flies carrying the destabilized X chromosome transmit a mutant allele not apparent in their phenotype (cryptic mutation). We are interested in learning about the origin and the underlying molecular mechanisms for transmission of cryptic mutations.

In our system, two X-linked loci exhibit a high frequency of cryptic mutation generation and transmission. These are cut wings (ct) and forked bristles (f) loci. In a particular cross, for example, more than 2% of males carrying the destabilized X chromosomes produce at least one sperm with cryptic ct. Our experimental data are consistent with the view that a transposable element or elements are involved in transmission of the mutations. A number of separate lines of evidence indicate that the ordinary meiotic recombination process is not likely involved in generating the cryptic mutations.

Suppressible insertion mutations, transposon-mediated instability, heteroduplex nature of cryptic mutant loci, and involvement of extrachromosomal replicating elements are some of the likely models we would like to test. Our experimental approach is to correlate genetic data with Southern blot transfer analysis and in situ hybridization.

Principal Investigator and All Other Personnel Engaged on the Project:

Johng K. Lim	Research Geneticist	LG	NIEHS
Burke H. Judd	Chief	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: If one of the two X chromosomes in a hybrid Drosophila female is the unstable X chromosome (Uc) or its derivatives, the other previously stable X chromosome becomes genetically unstable. This phenomenon was given a name homologue destabilization, and it was proposed that donation of a transposable element or elements by the Uc to the previously stable X chromosome is responsible for the acquired instability. The acquired genetic instability results in transmission of a mutant gene not apparent in the phenotype of individuals (cryptic mutation).

Two major approaches were employed in further study of the phenomenon: 1) tracing the transmission of cryptic mutations by breeding experiments, and 2) search for a transposable elements involved in the process by Southern analysis and in situ hybridization.

MAJOR FINDINGS AND PROPOSED COURSE: Use of special chromosomes enabled us to trace the transmission of cryptic mutations. It is now clear that not all of the X-linked loci can be destabilized by the system. For example, white (w) and vermilion (v) loci are highly refractory while cut wings (ct) and forked bristles (f) loci are readily responding to the destabilizing system. It has been shown that the Uc strain need not express a ct phenotype to generate a cryptic ct mutation in the destabilized X chromosomes. Experiments with inversion and flanking markers around the ct locus showed that ordinary meiotic recombination is not involved in the destabilization process. Finally, the experiments clearly showed that the acquired instability can be transmitted as a genetic trait.

The search for a transposable element led to detection and isolation of an insertion sequence from a ct mutant (ct^{512}) generated through the destabilization system. About 9 kb insertion sequence from the mutant has been cloned into a λ phage by Dr. Jack and was designated as 512-1. Currently the insert is being characterized by restriction mapping.

The results of preliminary in situ hybridization experiments, using the 512-1 as probe, indicate that the 512-1 sequence tends to move within the X chromosome. This observation is important because it is known that almost all of the chromosome breaks detected in the Uc-generated lethal mutations occur in the X chromosome. Whether 512-1 is, or is not, the transposable element responsible for the destabilization process is not clear at present. Well directed and designed in situ hybridization experiments supported by Southern blot transfer analysis may solve this problem. Such experiments and analysis are underway.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Cryptic mutations arising through homologue destabilization violate at least two basic tenets of genetics: the stability of genes and the structural integrity of alleles in hybrids. If a gene is so unstable that one cannot predict gametic genotype of an individual with a defined phenotype, Mendelian principles are utterly useless. Somewhat related to gene stability is structural integrity of genes in hybrids. Alleles in hybrids are supposed to maintain structural integrity and this is one of the fundamental assumptions necessary for predicting the genetic ratios. Homologue destabilization clearly show that one chromosome affects the integrity of its homologue which in turn leads to genetic instability.

Study of genetic instability is as old as the rediscovery of Mendel's principles. Yet, until recently, the phenomenon of genetic instability has been treated as rare exceptional events. Presence in all genomes of substantial amount of repetitive sequences and wide occurrence of transposable elements discovered through recombinant DNA techniques argue otherwise. it is important to understand the destabilization process and to determine whether it is a general phenomenon that may under some conditions occur in all eukaryotic organisms including humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61028-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutations Affecting the Expression of an RNA Polymerase II Locus in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lillie L. Searles Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, 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 *(Use standard unreduced type. Do not exceed the space provided.)*

RNA polymerase II is a complex, multisubunit enzyme which transcribes structural genes in eukaryotes. Recently, we cloned a gene which encodes one subunit of Drosophila RNA polymerase II. The goal of this research project is to define sequences that control the expression of this gene and to determine how insertions of a transposable element at the locus interfere with gene expression.

Principal Investigator and All Other Personnel Engaged on the Project:

Lillie L. Searles	Staff Fellow	LG	NIEHS
Robert A. Voelker	Research Geneticist	LG	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Sequences from the RNA polymerase II (RpII) locus were cloned initially from a mutant containing an insertion of the transposon, P element, at the locus. Subsequently, DNAs from wild type and a number of other mutants have been cloned. Mutant DNAs have been analyzed by "Southern" hybridization and more detailed information is being obtained by DNA sequencing.

MAJOR FINDINGS AND PROPOSED COURSE: From analyses of several P element insertion mutations that inactivate the RpII locus we have found that insertions of this transposable element are clustered at two sites, one within coding sequences and the other outside the structural gene. In the case where insertion occurred outside the structural gene, reversion or restoration of function to the locus occurs by both precise and imprecise excision of P element. We would like to determine the manner by which insertion outside the structural gene interferes with gene expression and therefore to determine (a) the location and nature of sequences that are important for RpII locus function and (b) whether the insertion of P element sequences actively or passively disrupts gene expression. Currently, I am sequencing DNAs from wild type, insertion mutants and revertants. The P element insertion site has been localized and is within a few nucleotides of a possible transcription initiation signal, suggesting that insertion may block transcription of the locus. Work is continuing to determine the nature of sequences remaining at the locus after imprecise excision of P element and the manner in which these sequences differentially affect expression of the locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In mammals, transposon-like elements have been implicated in the alteration of gene function resulting in the development of cancer in some cases and perhaps other disorders as well. Using Drosophila transposons as model systems, we may gain a better understanding of how mobile genetic elements affect gene expression.

PUBLICATIONS

Searles, L.L., Jokerst, R.S., Bingham, P.M., Voelker, R.A., and Greenleaf, A.L.: Molecular cloning of sequences from a Drosophila RNA polymerase II locus by P element transposon tagging. Cell 31: 585-592, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61029-01 LG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning and Characterization of the vermilion Locus of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lillie L. Searles Staff Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The vermilion locus of Drosophila encodes an enzyme, tryptophan oxygenase, which catalyzes one of the first steps in the synthesis of the brown eye pigment. This locus has been well-defined genetically for a number of years. We intend to clone the vermilion locus to study the structure of the gene and control of its expression. We are particularly interested in determining the nature of spontaneous mutations at vermilion that are suppressible by mutations at suppressor of sable; hopefully, these studies will help to elucidate the mechanism by which suppression occurs.

Principal Investigator and All Other Personnel Engaged on the Project:

Lillie L. Searles	Staff Fellow	LG	NIEHS
Robert A. Voelker	Research Geneticist	LG	NIEHS
Shu-Mei S. Huang	Biological Laboratory Technician	LG	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Several mutations at the vermilion locus of Drosophila are suppressible by mutations at suppressor of sable. All of the suppressible alleles are spontaneous in origin. It is likely that these mutations are due to insertion of a transposable element for two reasons. Firstly, the majority of spontaneous mutations in Drosophila are insertions of mobile elements. Secondly, by analogy, suppressor of Hairy-wing, su(Hw), has been shown to suppress some insertions of the gypsy element in Drosophila. However, most of the loci suppressed by su(Hw) are complex or have undefined gene products and are not available for studies of the mechanism of suppression. The vermilion locus is suitable for such studies. We therefore propose to clone the vermilion locus in order to determine the nature of suppressible mutations. The locus will be cloned from a mutant which we have identified that contains a P element insertion at vermilion. Transcribed regions will be identified by hybridization of polyadenylated RNAs to cloned DNA fragments. The sites of mutations which affect the locus will be determined and subsequently some mutants will be studied in more detail by, for example, measuring levels of vermilion transcript in wild type, mutant and suppressed strains, determining the site of transcription initiation in different strains and by DNA sequencing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the nature of spontaneous mutations and how these effects may be suppressed is an important step toward resolving problems related to genetic disorders in higher organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65021-11 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of Germinal Mutation Induction in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

F. M. Johnson, Ph.D./Research Geneticist/LGMM/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 Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. 1/83)

GPO 895-100

Principal Investigator and Other Professional Personnel Engaged on the Project:

F. M. Johnson	Research Geneticist	LGMM	NIEHS
L. C. Skow	Sr. Staff Fellow	LGMM	NIEHS
M. L. Snell	Bio. Lab. Tech.	LGMM	NIEHS

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 of 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.

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.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65022-03 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Types of Sperm Anomalies in Males after Treatment with a Mutagen

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

F. Binkert, Ph.D./Visiting Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to elucidate the connection between mutagenic treatment and type of sperm anomalies. Different types of anomalies were tested for their usefulness in mutagenic screening. Different doses of methyl methanesulfonate (MMS), ethylnitrosourea (ENU), procarbazine, ethidium bromide, and acriflavine served as inducers.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

F. Binkert	Visiting Fellow	LGMM	NIEHS
H. V. Malling	Section Head	LGMM	NIEHS
J. G. Burkhart	Chemist	LGMM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Basically we used sperm smears of single mice. A combined procedure enabled us to detect morphological and enzymatic abnormalities. The enzyme content was measured by eye and by absorbance readings with a Zeiss Universal. Some results will be substantiated by electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: In the study of DBA/2J mice treated with methyl methansulfonate (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 acriflavine 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. Enzyme activity could be influenced in 3 ways: ENU and procarbazine decreased activity mainly in differentiating spermatogonia and spermatocytes; acriflavine and ethidium bromide in late spermatocytes and early spermatids; MMS had no influence. All studies are in the preparation process for publications.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the view of mutagenicity, germ cells are the target of most concern. Sperm are the endproduct of the male germ cell line. They are easily available in large numbers. This research project enlarges the fundamental knowledge of the presently used sperm anomaly test (only shape of sperm head evaluated). The additional evaluation of other abnormalities, e.g., mitochondrial damage, will improve the utility and application of sperm as test objects.

PUBLICATIONS

Binkert, F., Burkhart, J. G., and Malling, H. V.: A new sperm abnormality test using enzymatic staining. Environmental Mutagenesis, 4: 324 (1982).

Burkhart, J. G., Ray, C. P., and Malling, H. V.: Effect of procarbazine treatment of mice on α-glycerophosphate dehydrogenase activity and frequency of selected abnormalities in sperm. Mutation Res., 92: 249-256 (1982).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65023-03 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Monoclonal Antibodies to Detect Mutant Forms of LDH-C in Sperm

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

L. L. Wright, Ph.D./Sr. Staff Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

Comparative Medicine Branch; Biometry Branch

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Pursuant to these goals, we have discovered that LDH-A (muscle), LDH-B (heart, and LDH-C (testes) are immunochemically cross-reactive. The degree of cross-reactivity is dependent upon the species from which (a) the immunogen is purified, (b) antisera are derived, and (c) LDH employed in the assay is purified. A number of investigators have been studying LDH-C for its potential use as a contraceptive vaccine. Our studies show, if LDH-C is employed as a vaccine, it may lead to autoimmune complications.

Principal Investigator and All Other Personnel Engaged on the Project:

L. L. Wright	Senior Staff Fellow	LGMM	NIEHS
J. G. Burkhart	Chemist	LGMM	NIEHS
J. H. Swofford	Bio. Lab. Tech.	LGMM	NIEHS
R. A.. Schmaltz	Biol. Aid	LGMM	NIEHS

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 of 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 SP2/0-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 72 hybrids which produce antibody that are cross-reactive to mouse and rat LDH-C. Unfortunately these hybrids also have considerable background activity in our assay. We are presently attempting to clarify this background activity, either by fine-tuning our assay or by employing other assays, such as radioimmune assays to characterize these hybrids and reduce background activity. We have produced clones which are reactive specifically to LDH-C. We have characterized two clones which produce antibodies which are cross-reactive to mouse and rat LDH-C. As measured by EIA, we have another two clones which are

reactive specifically to rat LDH-C. We intend to increase our panel of monoclonal antibodies known to be reactive to isomeric forms of rat LDH-C. These antibodies 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.

Pursuant to the aforementioned course of study, we have accidentally discovered exciting immunochemical activity that exists among LDH A, B, and C. It has been reported that LDH C is not immunochemically cross-reactive with somatic forms of LDH purified from heart (LDH B) or muscle (LDH A). Based on this premise, LDH C has been considered for its potential as a contraceptive vaccine. We have discovered that mouse heterologous antisera to either mouse or rat LDH C are cross-reactive with LDH A and B purified from muscle and heart tissues of mice. Interestingly, rabbit antisera to mouse LDH C are not cross-reactive to either mouse LDH A or B. Thus, the degree of cross-reactivity is dependent upon the species from which the (a) immunogen LDH C is purified, (b) antisera are derived and (c) LDH employed in the assay is purified. The determination that LDH A, B, and C are immunochemically cross-reactive has serious implications. When employed as an immunologic approach to contraception, LDH C may cause autoimmune complications. It remains to be determined if these antibodies may react in vivo as autoantibodies to native forms of mouse LDH A and B. Studies are in progress to determine if autoantibodies promote tissue damage in mice immunized with mouse LDH C.

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.

PUBLICATIONS

Wright, L.L., Swofford, J.H., Burkhart, J.G., Skow, L.C., and Malling, H.V. (1983) Evidence of immunochemical cross-reactivity among lactate dehydrogenases A, B, and C (X). Isozyme Bulletin (abst.), in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65024-03 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

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

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

F. Binkert, Ph.D./Visiting Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to elucidate the inheritance of different sperm abnormalities found in the BALB/cJ and PL/J mouse strains. Reciprocal crosses between the strains BALB/cJ and DBA/2J, as well as between BALB/cJ and PL/J, were performed. In addition, a wide range of backcrosses were completed. The unveiling of the complex results in a blind evaluation of coded slides is still underway in the Statistics Branch.

Principal Investigator and All Other Personnel Engaged on the Project:

F. Binkert	Visiting Fellow	LGMM	NIEHS
H. V. Malling	Section Head	LGMM	NIEHS
J. G. Burkhart	Chemist	LGMM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Among the various inbred mouse strains, PL/J and BALB/cJ have a high level of sperm abnormalities. The various types of sperm abnormalities are elucidated by using a stain for proteins and one for the mitochondrially bound enzyme α-glycerophosphate dehydrogenase. The mode of inheritance is studied between the strains PL/J, BALB/cJ and DBA/2J on crosses and backcrosses, as well as comparisons to the C57BL/6J strain and the recombinant inbred lines CXB from Bailey.

MAJOR FINDINGS AND PROPOSED COURSE: The four inbred strains, BALB/cJ, PL/J, DBA/2J, and C57BL/6J have a sperm abnormality pattern which is clearly distinct from each other. After creating a discriminant function for each strain, every single animal mapped to its own strain. The F_1 generation of crosses between BALB/cJ (B) and DBA/2J (D) showed a recessive trait with a very weak penetrance of the mutant character. But again, after creating a discriminant function of BxD and DxB_1, every animal always was mapped into its own cross. The offspring of crosses between BALB/cJ (B) and PL/J (P) had, in comparison to P, a reduced rate of abnormal sperm. The rate, however, was higher than among the offspring from crosses between D and B. There was a clear-cut maternal effect for the PL abnormality by the P strain. The animals of the reciprocal crosses could again be separated by discriminant functions. Analyses of the complex backcross data, done until now, did not lead to a final result. When all statistics are done, the study will be processed for a publication.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65027-02 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Genetics of the Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

L. C. Skow, Ph.D./Senior Staff Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. 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 background by repeated back-crossing.

Principal Investigator and Other Professional Personnel Engaged on the Project:

L. C. Skow	Senior Staff Fellow	LGMM	NIEHS
F. M. Johnson	Research Geneticist	LGMM	NIEHS
K. K. Dugger	Biological Aid	LGMM	NIEHS
M. L. Snell	Biol. Lab. Tech.	LGMM	NIEHS

PROJECT DESCRIPTION

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65028-02 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Mutations in Mouse Globin Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

L. C. Skow, Ph.D./Senior Staff Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 HBA and HBB. Multiple independently induced and/or spontaneous variants for each of these genes are analyzed by DNA probes produced from the normal (wild-type) mRNA or DNA for aberrant gene structure, processing, and expression. Analysis of a naturally occurring β-globin deficiency has recently been completed and the mutation shown to be a small deletion affecting only the β-globin gene. The mutation is designated Hbb^{th-1} for β-thalassemia and represents the only non-human β-thalassemia yet discovered. Biochemical analysis of an induced α-globin mutation, done in collaboration with Dr. R. Popp, Oak Ridge National Laboratory, revealed an amino acid substitution at $\alpha 89^{His\ Leu}$, likely produced by a transversion type of base substitution.

Principal Investigator and Other Professional Personnel Engaged on the Project:

L. C. Skow	Senior Staff Fellow	LGMM	NIEHS
B. A. Burkhart	Biologist	LGMM	NIEHS
F. M. Johnson	Research Geneticist	LGMM	NIEHS

PROJECT DESCRIPTION

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 couterpart 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.

PUBLICATIONS

Skow, L. C., Burkhart, B. A., Johnson, F. M., Popp, R. A., Popp, D. M., Goldberg, S. Z., Anderson, W. F., Barnett, L. B., and Lewis, S. E.: A mouse model for β-thalassemia. Cell, submitted.

Popp, R. A., Bailiff, E. G., Skow, L. C., Johnson, F. M., and Lewis, S. E.: Analysis of a mouse α-globin gene mutation induced by ethylnitrosourea. Genetics, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65029-02 LG

PERIOD COVERED

October 1, 1982 through September 30, 1982

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Mouse Lens Mutations

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

L. C. Skow, Ph.D./Senior Staff Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 nine mouse eye mutants have 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. cDNA probes for each of the classes of mouse lens crystallins have been obtained from J. Piatigorsky, National Eye Institute, and are being used to screen inbred strains of mice for restriction polymorphisms which will permit mapping of the mouse crystallin genes. This information will be used to facilitate genetic analysis of the lens mutants and to identify those types of lens mutants which are likely to have defective crystallin genes. We have successfully developed surgical and electrophoretic techniques to permit the incorporation of lens tissue into the biochemical specific locus test being conducted by Dr. Susan Lewis at the Research Triangle Institute. The use of lens tissues contributes an additional 13 loci to the test and also provides for the detection of mutations which produce microphthalmias and/or cataracts.

Principal Investigator and Other Professional Personnel Engaged on the Project:

L. C. Skow	Senior Staff Fellow	LGMM	NIEHS
K. K. Dugger	Biological Aid	LGMM	NIEHS
B. A. Burkhart	Biologist	LGMM	NIEHS

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 -crystallins. Exp. Eye Res. 34: 509-516 (1982).

Skow, L. C, Popp, R. A. and Bailiff, E. G.: Identification of a second lens crystallin variant, LEN-2, in the mouse. Biochemical and genetic analysis of LEN-1 and LEN-2. Exp. Eye Res., submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65030-02 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutation of the Murine and Human Major Histocompatibility Complex Loci

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. J. Hessling, Ph.D./Staff Fellow/LGMM/NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

J. J. Hessling	Staff Fellow	LGMM	NIEHS
H. V. Malling	Section Head	LGMM	NIEHS
L. L. Wright	Senior Staff Fellow	LGMM	NIEHS
J. G. Burkhart	Chemist	LGMM	NIEHS

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 athymic 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 papain to obtain the F(ab) 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-labeled 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, both, 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 induced 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 will 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. The monoclonal antibody preparations have been assayed for strength and specificity on splenocytes of inbred mouse strains which differ at the MHC locus and on target cell lines mentioned above. Each preparation demonstrated high titers and specificity as published in the literature. However, attempts to utilize these antibodies in a dual-label detection assay,

which would screen for lost mutations at the MHC locus, have thus far been unsuccessful. Indirect immunofluorescence assays, using unlabeled monoclonal antibodies of different subclasses and several pairs of either fluorescein-labeled or rhodamine-labeled secondary antibodies directed against each subclass, did not maintain the specificity required due to interactions between the secondary antibodies. It will be necessary to find pairs of secondary antibodies which will not react with each other. Direct immunofluorescence assays, using pairs of monoclonal antibodies labeled with either fluorescein or rhodamine, have not been possible, since none of the numerous antibody preparations which were directly labeled with fluorophores retained titers necessary to be useful in an immunofluorescence assay. This was probably due to the frequently observed lability of monoclonal antibodies, in particular, to enzymatic degradation and coupling procedures. More gentle procedures will be required and attempts may be made to label whole antibodies rather than Fab fragments in order to increase the probability of maintaining active preparations.

Single-label immunofluorescence assays which would screen for gain mutations at the MHC locus have been performed utilizing both fluorescence microscopy and flow cytometry. Data from fluorescence microscopy has not been analyzed yet but it has been determined that the fluoresence-activated cell sorter, as it is currently designed, detects a non-specific background level which is too high to allow its use for mutation assays. A slit-scan modification may eliminate this non-specific background. Preliminary experiments to determine the sensitivity of the fluorescent technique in the fluorescent microscope 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.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65031-01 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Incorporation of ϕX174 into Mouse Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

H. V. Malling/Section Head/LGMM/NIEHS

COOPERATING UNITS *(if any)*

Department of Bacteriology and Immunology
University of North Carolina at Chapel Hill

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, NC

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was undertaken to study site specific mutagenesis in mammals. Mouse L cells (tk-/-) were co-transformed with double stranded RF DNA from ϕX174 am_3 CS70 (am_3 - amber mutation; CS70 - cold temperature sensitive mutation) and λ-phage containing the genomic $tk^{+/+}$ gene. The transformed cells were selected on HAT medium and single colony isolates were made. One cell culture, T15-1.3, was analyzed for its content of X174 DNA by digestion with restriction enzymes, electrophoresis, southern blot, and probing with nick translated ϕX174. Several bands were visible, the strongest band co-migrated with ϕX174 DNA. The level of radioactivity indicated approximately 3-10 copies of ϕX174 double stranded DNA in this band. The DNA from the transformed L-cells were digested with the restriction enzyme pst-1 and ligased with T_4 ligase. This DNA was transfected into E. coli spheroplasts and ϕX174 plaques were obtained from the transformed L-cells.

Principal Investigator and All Other Personnel Engaged on the Project:

H. V. Malling	Section Head	LGMM	NIEHS
J. G. Burkhart	Chemist	LGMM	NIEHS
M. C. Fater	Biological Aid	LGMM	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mouse L-cells were transformed with double stranded RF forms of ϕX174 am_3 cs_{70}. Cultures were established from single colony isolates of the transformed cells. A technique was developed for storing frozen spheroplast from E. coli. These spheroplasts are used for transfection with ϕX174 prepared from DNA isolated from the transformed L-cells. Reverse mutations will be detected in am_3 by plating on E. coli strain C and in cs_{70} by incubating the plates at 27°C.

MAJOR FINDINGS AND PROPOSED COURSE: Preparation of spheroplasts are laborious and time consuming and the competence of the spheroplasts varies considerably between preparations. In order to circumvent this problem, a technique was developed to freeze spheroplasts while still preserving their competency. The spheroplasts maintain their competency for at least nine months. DNA from mouse L-cells transformed with ϕX174 was digested with the restriction endonuclease pst-1 and treated with T_4 ligase. This DNA was used for transfection of spheroplasts and ϕX174 plaques were recovered from this DNA. We will develop techniques for isolating the ϕX174 DNA from mammalian cells and in the future treat the L-cells transformed with ϕX174 am_3 cs_{70} with various mutagens. Reverse mutations of the am_3 and the cs_{70} will be measured among the phages recovered from DNA from treated and untreated cell cultures. In collaboration with the scientists at UNC, attempts are being made to transfect fertilized mouse eggs with ϕX174 which hopefully will result in mice with ϕX incorporated into their genome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This program may result in a system where site specific mutagenesis can be measured in any organ of a mouse from which DNA can be obtained in sufficient quantities. If successful, it may also enable use to understand mutation fixation in mammalian genome. It may also give very precise data of the sensitivity of the various sperm stages to mutagenic treatment. Knowledge of parameters about mutagenesis in a mammal is likely to give information about the damage induced by exposing the mammalian organism to a mutagen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65032-01 LG

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Induced Mutations at the MOD-1 and GPI Loci of Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James G. Burkhart/Chemist/LGMM/NIEHS

COOPERATING UNITS *(if any)*

Research Triangle Institute

LAB/BRANCH

Laboratory of Genetics

SECTION

Mammalian Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several mutations have been induced by n-ethyl-n-nitrosourea in mice and detected by the biochemical specific locus test at the MOD-1 and GPI loci. The frequency of MOD-1 mutations is much greater than the frequency of GPI mutations. The objectives of this work are to (1) complete a comparative biochemical analysis of induced mutations of MOD-1 and GPI in mice, (2) analyze at the molecular level the relationship of sequence, chromatin structure, and regulation to the frequency and location of induced DNA lesions, and (3) evaluate whether or not any of the induced mutations can be used as markers for mutational events directly in exposed animals.

Principal Investigator and All Other Personnel Engaged on the Project:

J. G. Burkhart	Chemist	LGMM	NIEHS
H. V. Malling	Section Head	LGMM	NIEHS
F. M. Johnson	Research Geneticist	LGMM	NIEHS
L. C. Skow	Sr. Staff Fellow	LGMM	NIEHS
J. Benziger	Bio. Lab. Tech.	LGMM	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: MOD-1 and GPI have been purified from normal DBA/2J mice and antibodies to each produced in rabbits. One MOD-1 electrophoretic mobility variant has been purified and injected into rabbits. Two apparent nulls of MOD-1 have been found to produce low levels of enzymatically active protein that is identifiable by antibody to normal DBA/2J MOD-1. Further biochemical and genetic analysis will establish whether these mutations are structural or regulatory. Additional mutations at each loci will be biochemically and immunologically characterized. Probes for molecular analysis will be produced by screening a cDNA library for clones by antibody selection and/or unambiguous synthetic oligonucleotides produced form protein sequence data. Antibody selection techniques for clones producing antigenic polypeptides have been developed such that less than 50 pg of MOD-1 subunits can be detected. The cDNA sequences will be used to analyze genomic libraries produced from DNA of mutant animals. Specific induced abnormal proteins will be carefully evaluated for their potential use as biochemical markers of induced mutations in whole animals with detection by antibodies. Antibodies will then be made specific for antigenic sights of the abnormal proteins. 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 as 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.

LABORATORY OF MOLECULAR BIOPHYSICS

LABORATORY OF MOLECULAR BIOPHYSICS
SUMMARY STATEMENT

The Laboratory of Molecular Biophysics was formed by combining the Molecular Biophysics Workgroup of the Laboratory of Environmental Biophysics and the Laboratory of Environmental Chemistry. The goals of the Laboratory of Molecular Biophysics are to develop, improve and utilize spectroscopic methods to monitor the molecular interactions that occur between environmental agents and biological systems, to develop, improve and utilize analytical methodology for specified chemical agents, and to conduct biochemical, physical organic and bio-organic studies of environmental agents and their conversion products with emphasis on biomechanism elucidation. The Laboratory is organized into four separate Workgroups: Molecular Biophysics, Analytical Chemistry, Nuclear Magnetic Resonance, and Bio-organic Chemistry.

MOLECULAR BIOPHYSICS

The Molecular Biophysics Program is concerned with understanding, at the molecular level, the interaction of environmental agents with target biological systems, including nucleic acids, proteins and membranes. For these studies a number of highly sophisticated spectroscopic techniques (electron spin resonance, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry) are employed. Particular emphasis is placed on the detection and identification of reactive free radicals (including active oxygen species) generated during the metabolism of environmental chemical agents. There is now convincing evidence that free radicals are involved in a number of pathologic conditions including chemically induced carcinogenesis, pulmonary fibrosis, methemoglobinemia and hemolytic anemia.

Before free radicals can be clearly implicated in any toxic effect, the free radical metabolite must be demonstrated to exist under appropriate biological conditions. During the past year free radicals have been detected during the one-electron oxidation of sulfur dioxide, benzidine, tetramethylhydrazine and acetaminophen by horseradish peroxidase, lactoperoxidase and prostaglandin hydroperoxidase. The reduction of a variety of nitroaromatic drugs, including nifurtimox, nitrofurantoin, misonidazole, and metronidazole, by microsomal and mitochondrial fractions of rat liver has also been investigated. Under anerobic conditions the corresponding nitro anion radical was detected in all cases by electron spin resonance. The site of nitro reduction by mitchondria appeared to be NADH dehydrogenase and outer membrane NAD(P)H cytochrome c reductase. Under aerobic conditions the nitro anion radicals react with oxygen to yield superoxide. In other experiments, evidence was obtained for the P-450 mediated reduction of sulfur dioxide, oxygen and gentian violet to free metabolites.

Many chemical agents are known to cause photosensitization of exposed tissues such as the skin and eyes. The chemical agent may be endogenous (eg. protoporphyrin), a drug (eg. p-aminobenzoic acid in sunscreens, salicylanilides in soaps) or an environmental agent (eg. polycyclic aromatic hydrocarbons in coal tar). 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 still unknown. Current work is concerned with the role played by free radicals in photosensitization. The photochemistry of benoxaprofen, an anti-inflammatory drug that causes acute phototoxicity in many patients, and related benzoxazoles

has been studied in both aqueous and organic solvents. Evidence for photoionization and photohomolysis were obtained. Under aerobic conditions both singlet oxygen and superoxide were detected. A photolysis study of musk ambrette (2,6-dinitro-3-methoxy-1-methyl-4-t-butylenzene), a common component of perfumes and soaps, revealed the presence of two nitro anion radicals during irradiation. In the presence of oxygen superoxide was detected. A comparison of musk ambrette with two related musk compounds, musk ketone and musk xylene, suggested that planar nitro anion radicals may be responsible for cutaneous photosensitization caused by these agents.

Preliminary studies have been completed on anthracene and fluoranthene which are the two main components of coal tar. Studies in mice have been shown that anthracene is photocarcinogenic, while fluoranthene is known to be a cocarincogen. Both compounds caused a concentration dependent photohemolysis of human erythrocytes that was markedly enhanced by oxygen. Photohemolysis was accompained by a decrease in the α-helical content of red cell membranes and extensive crosslinking of spectrin and other membrane proteins. Singlet oxygen has been implicated in these phenomena.

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 have usually 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 predicitve abilities in analytical applications of mass spectrometry and to study the intrinsic chemistry applications of ionic species.

Specific accomplishments include the demonstration that positive ions produced by charge reversal of negative ions gave the same collisional activation spectra of positive ions of the same structure produced by conventional means, thus validating this technique for the study of gas phase ion structure. A focused secondary ion mass spectrometer (SIMS) source has been designed, constructed and evaluated for the analysis of polypeptides. A factor of 10 gain in minimum detectable quantity was realized. Tandem mass spectrometry (TMS) was compared with GC/MS for typical environment samples. Although GC/MS was found to be more time-consuming it did give more reliable results. The HPLC/MS system has been upgraded to include a microbore HPLC so that the previous 100:1 sample split is now avoided. The effect of various HPLC parameters on the observed mass spectra has been studied.

NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

Installation of a high field superconducting NMR spectrometer operating at 360 MHz has been completed. This instrument will increase our capability to solve biological problems by providing greater chemical shift dispersion, greater sensitivity for various nuclei and the ability to use large sample tubes. A permanent full time NMR spectroscopist has been recruited to supervise the operation of the NMR facility. Future work in this area will include studies on the solution conformation of peptides of biological importance, conformational studies on metabolites of polycyclic aromatic hydrocarbons and in vivo and in vitro metabolic studies on environmental chemical agents.

BIO-ORGANIC CHEMISTRY

The Laboratory's bio-organic chemistry program is concerned with the development of methodologies to detect and identify metabolites of environmental chemicals in biological systems. Research is also carried out on the mode of interaction of environmental agents with biological systems at the molecular level with particular emphasis on metabolic factors. Work has continued on the transport and metabolism of phthalate esters. Studies have shown that while the phthalate moiety of diethylhexyl phthalate (DEHP) does not bind to macromolecules, a portion of the ethylhexyl chain does interact with DNA in vivo. Phthalate has been detected in each of 200 samples of human urine, thus confirming ubiquitous exposure to this agent. Metabolic investigations have shown that DEHP metabolism involves extracellular, microsomal, cytoplasmic and mitochrondrial enzyme systems. In the rat over 24 metabolites have been identified. However, a comparison of the relative levels of ten major metabolites in seven animal species indicates wide species differences in DEHP metabolism. An important finding of this work is that the rat is a very poor model for man. Future studies will focus on the isolation, identification and characterization of the enzyme systems involved in DEHP metabolism.

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. Investigations of structure-toxicity relationships in polyhalogenated aromatic hydrocarbons are continuing to use the guinea pig as an extremely sensitive animal model. Lethality in guinea pigs appears to be associated with toxic planar (or coplanar) compounds with potentiation possible by certain favorably substituted halogenated biphenyls. This differs from the structure-activity relationship associated with the cytosol binding proteins (dioxin receptor). A model, based on polarizabilities, is being developed that will account for the observed differences. A protein binding model is being developed which it is hoped will show a better correlation with the observed structure-toxicity results. The exact nature of this binding is being studied using X-ray crystallography, molecular graphic and theoretical chemistry approaches. Other work will attempt to show the relationship of such binding to the mechanism of dioxiin and related compound toxicity.

The Laboratory's organic chemistry program develops new and improved methods in synthetic chemistry to support diverse studies on biomechanism 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 metabolites 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 enzyems. The idea 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 tranferase reaction underscore the relevance of stereochemical factors in the influencing the rate of elimination of enantiomeric oxides via the glutathione pathway. Specific accomplishments include a practical synthesis of N-acetylcysteine adducts of alkene and arene oxides. Analytical conditions have now been developed for the separation of diastereomeric glutathione adducts of various alkene and arene oxides using high pressure liquid chromatography. The use of optically pure epoxides of known absolute stereochemistry has allowed configurational assignments for several of these glutathione adducts. The stereochemical profiles made available by these methods are being applied towards the elucidation of the mechanism of catalysis by the glutathione transferase enzymes.

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 the bombesin series. The polypeptide bombesin may serve as a mark for small cell lung cancer. For the glutathione conjugates of (±)-styrene oxide and (±) benzo[a]pyrene-4,5-oxide the four diastereomers derived form each epoxide are reproducibly separated. For (±) benz[a]anthracen-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. The important role played by hydrophobicity in the separation of peptides by reverse-phase liquid chromatography has been exploited in the analysis of bombesin and related peptides. An experimental paramater αpH has been developed which reflects changes in the intrinsic hydrophibicity of a peptide as a function of pH and organic solvent. The αpH values were found to correlate well for peptide homologs.

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 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 methodlogy 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 benzoanthracenes and benzoanthracene derivatives capable of exhibiting optical activity. Functionalized benzoanthracenes with the potential for conversion into other polynuclear aromatic hydrocarbons have been synthesized. Known and hyopthetical oxygenated metabolites of some of these hydrocarbons have been prepared. Finally heteroaromatic polynuclear hydrocarbons have been synthesized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10003-04 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthetic and Analytical Studies in Bioorganic Chemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Oscar Hernandez Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Behavioral and Neurological Toxicology

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Oscar Hernandez	Senior Staff Fellow	LMB	NIEHS
M.B. Gopinathan	Visiting Fellow	LMB	NIEHS
L. Lazarus	Research Chemist	LBNT	NIEHS

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: Analytical conditions were developed for the separation of diastereomeric glutathione adducts of (±)-styrene oxide, (±)-benzo[a]pyrene 4,5-oxide, (±)-benz[a]anthracene 5,6-oxide, pyrene 4,5-oxide, and phenanthrene 9,10-oxide. The methodology developed involved reverse-phase HPLC and a combination of pH and solvent effects designed to enhance structural differences among thioether diastereomers of a given epoxide. The availibility of diastereomerically pure glutathione conjugates derived from optically pure styrene oxide(s), benzo[a]pyrene 4,5-oxide, and benz[a]anthracene 5,6-oxide enabled us to assign absolute configuration to individual peaks on the HPLC trace. Stereochemical profiles for these epoxides became available through this work and are now being applied to the elucidation of reactions mechanisms catalyzed by the glutathione transferases. Of special interest is that, within a given set of regioisomers, the diastereomer with the S-configuration at the carbon-bearing sulfur emerges earlier than the corresponding R-regioisomer.

The role of hydrophobicity in the separation of peptides by reverse-phase HPLC was exploited in the analysis of bombesin and related peptides. The intrinsic hydrophobic character of peptides (10-14 residues) was altered as a function of pH. Changes in elution were correlated to the presence of a histidine residue in a hydrophobic environment. The effect of solvent was also explored and conditions evolved out of this study which were found diagnostic for the analysis of bombesin. An experimental parameter, αpH, was defined as the positive quotient of capacity factors at pH 4 and pH 7. Peptide homologs were found to give identical αpH values for a given solvent system.

Ongoing projects include the extension of the αpH concept to other peptide families and the determination of αpH values at high pH with the aim of establishing a potential correlation between capacity factor and the presence of lysine and arginine residues.

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

Erisman, M.D., Linnoila, R.I., Hernandez, O., DiAugustine, R.P. and Lazarus, L.H.: Envidence of bombesin in a human oat-cell carcinoma. Proc. Natl. Acad. Sci. U.S.A., 79: 2379-2383, 1982.

Lazarus, L.H., Di Augustine, R.P., Jahnke, G.D. and Hernandez, O.: Physalaemin: an amphibian tachyranin in human lung small-cell carcinoma. Science 219: 79-81, 1983.

Hernandez, O., Bhatia, A.V. and Walker, M.P.: HPLC separation of the diastereomeric glutathione adducts of styrene oxide. J. Liquid Chromatogr., (in press).

Hernandez, O. and Kohli, K.K.: Removal of the nonionic detergent Emulgen 911 from solubilized microsomes by HPLC. J. Liquid Chromatogr. (in press).

Hernandez, O., Bhatia, A.V. and Walker, M.P.: HPLC separation of diastereomeric adducts of glutathione with some K-region arene oxides. J. Liquid Chromatogr. (in press).

Walther, H.J., Parker, C.E., Harvan, D.J., Voyksner, R.D., Hernandez, O., Hagler, W.M., Hamilton, P.B. and Hass, J.R.: Analysis of aflatoxins and reaction products of sodium bisulfite by fast atom bombardment mass spectrometry. J. Agr. Food Chem. 31: 168-171, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10004-04 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Nuclear Magnetic Resonance (NMR) Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James D. McKinney Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

NMR ^{1}H chemical shifts have been determined for a series of N-acetylcysteine derivatives of alkene and arene oxides. ^{1}H and ^{13}C studies of ^{13}C enriched compounds have permitted structural assignments in isomeric mixtures.

Principal Investigator and All Other Personnel Engaged on the Project:

James D. McKinney	Research Chemist	LMB	NIEHS
Oscar Hernandez	Visiting Scientist	LMB	NIEHS

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: The ^{1}H-NMR spectra of a series of mercapturic acid derivatives of alkene and arene oxides have been determined. The alkene oxides included: styrene oxide, α-methylstyrene oxide, β-methylstyrene oxide, and 1,2,3,4-tetrahydronaphthalene-1,2-oxide; arene oxides included: phenanthrene 9,10-oxide (K-region), and naphthalene 1,2-oxide (non K-region). The thioether derivatives analyzed by ^{1}H-NMR were single diastereomers. Chemical shift differences for diastereomers having the same configuration at the carbon-bearing sulfur showed a similar pattern, which may prove diagnostic for assignment of stereochemistry.

Work is nearing completion on a 1-^{13}C labeled hexabromonaphthalene mixture prepared from 1-^{13}C labeled naphthalene. Both the proton and carbon NMR spectra of this mixture were diagnostic for assignment of the bromine substitution patterns in combination with other spectral and chromatographic data on the mixture. Positive structural assignment of the isomers in the mixture was essential to understanding their biological properties.

Projects in progress include: 1) examination of the ^{33}S-NMR of diastereomerically pure N-acetylcysteine adducts of alkene and arene oxides; 2) ^{1}H-NMR of glutathione conjugates of arene oxides; 3) conformational analysis of glutathione conjugates of arene oxides; 4) conformational analysis of the peptide bombesin, particularly the pH dependence in attempts to explain the behavior of this peptide under reversed-phased HPLC conditions.

Qualified NMR spectroscopist has been identified and approved by Intramural Research Council; negotiations toward recruitment continue.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NMR spectroscopy is probably the single 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 environmental 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10007-03 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High Pressure Liquid Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Carol E. Parker Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Research Services Branch

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 the analysis of thermally labile compounds.

Principal Investigator and All Other Personnel Engaged on the Project:

Carol E. Parker	Research Chemist	LMB	NIEHS
J. Ronald Hass	Research Chemist	LMB	NIEHS

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. A Gilson micro HPLC system has been purchased and is presently being installed and tested.

MAJOR FINDINGS: The system is now working in both positive and negative ion chemical ionization modes. A similar interface has been designed and used for LC/MS/MS.

The dependence of signal intensity and degree of fragmentation in direct liquid introduction liquid chromatography/mass spectrometry (DLI-LC/MS) on source pressure, solvent composition, and temperature was demonstrated. The results showed that the sample response varies with pressure and that optimum source pressure varies with solvent composition. This causes some difficulty in maintaining optimal operating conditions in a gradient elution analysis. Varying the solvent composition has no affect on protonated molecular ion sensitivity; rather it changes the amount of fragmentation observed. The extent of fragmentation also depends greatly on temperature. The use of solvent cluster ions and source pressure as a rapid method of optimization of the DLI probe position was examined.

A tandem quadrupole mass spectrometer was used to identify ion-molecule reaction products in two common reversed-phase solvents, acetonitrile/water and methanol/water. The collision-induced dissociation spectra of normal and isotopic labeled solvents and high-resolution data provide the information to deduce the empirical formulas and structures for the cluster ions. The solvent effect on sample protonation and fragmentation was discussed.

A study has been initiated on the effects of the negative chemical ion (NCI) mass spectra of additions of chlorinated solvent modifiers to the HPLC solvent. Preliminary results indicate that the spectra produced show a great deal of similarity to reported chlorideattachment NCI mass spectra. In general, overall sensitivity is reduced, but spectra give more structural information in this chloride-attachment mode.

A micro HPLC system has been ordered, and parts of it are on site. The micro LC system appears to be functioning in the isocratic mode. Work is underway to develop gradient capabilities on this system.

PROPOSED COURSE: Work is continuing to determine the effect of source geometry, temperature, pressure and LC solvent systems, including chlorinated solvents for chloride-attachment NCI, on the sensitivity and specificity of LC/MS. 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, such as biological conjugates and metabolites of DES.

PUBLICATIONS

Voyksner, R.D., Parker, C.E., Hass, J.R., and Bursey, M.M.: The effects of pressure, temperature, and solvent ratio in an analysis by direct liquid introduction liquid chromatography/mass spectrometry. Anal. Chem. 54: 2583-2586, 1982.

Voyksner, R.D., Hass, J.R., and Bursey, M.M.: Analysis of the chemical ionization reagent plasma in liquid chromatography/mass spectrometry by direct liquid introduction mass spectrometry/mass spectrometry. Anal. phem. 54: 2465-2470, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10011-02 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methods for Determination of Polybrominated Biphenyls in Animal Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James D. McKinney Supervisory Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. No additional work done this reporting period. Project has been terminated.

Principal Investigator and All Other Personnel Engaged on the Project:

James D. McKinney Supervisory Research Chemist LMB NIEHS

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. [2]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: No additional work was done on this project. Project has been terminated.

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. Anal. Chem., 54: 1866-1871, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10012-01 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design of Laboratory Data Management System

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Mike P. Moorman Biomedical Engineer LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the design of a computer based data management system to service an analytical laboratory. This system supports three general functions, the control of instrumentation, the efficient distribution of data, and the facilitation of administrative tasks.

Principal Investigator and All Other Personnel Engaged on the Project:

Mike P. Moorman	Biomedical Engineer	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Requirement Analysis, Structured Systems Analysis, Structured Decomposition, Modular Design.

MAJOR FINDINGS AND PROPOSED COURSE: The general design is a radial network with most common functions implemented at the central node. A central computer is loosely connected to the micro computer based data systems, controlling each instrument thus allowing access to data from all instruments at a single point. Data distribution is achieved via common carrier to remote graphics terminals and remote micro computer implementations of the data manipulation system.

The principle control problem is the extension of an existing mass spectrometer data system to control multiple sector instruments.

Administrative tasks are performed by an office data system installed on the central computer and accessed by remote users via common carrier.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The accurate and timely distribution of meaningful data is a critical function in the analytical laboratory environment. The careful design of data manipulation and instrumentation control systems is necessary in order to realize the full potential of state of the art analytical instrumentation.

PUBLICATIONS

Van Stee, E.W., Boorman, G.A., Moorman, M.P., Sloane, R.A.: Time-varing concentration profile as a determinant of the inhalation toxicity of carbon tetrachloride. J. Tox. Envir. Hlth. 10: 785-795, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-12 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analytical Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Phillip W. Albro Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project area is to develop and define methodology for the qualitative and quantitative 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 reproducible extraction, cleanup, and accurate quantitation of chlorinated dibenzo-p-dioxins, its cytoplasmic binding protein, and other halogenated aromatic compounds. Major matrices studied have included milk and soil.

Principal Investigator and All Other Personnel Engaged on the Project:

Phillip W. Albro	Research Chemist	LEC	NIEHS
James R. Hass	Research Chemist	LEC	NIEHS
Y. Tondeur	Visiting Fellow	LEC	NIEHS
Kun Chae	Chemist	LEC	NIEHS
Carol Parker	Chemist	LEC	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods are generally developed using gas-liquid, thin-layer, open column and high pressure liquid chromatography, spectrophotometry (IR, UV, Visible, Fluorescence), mass spectrometry, and isotopic methods. Validation is in terms of recovery and reproducability.

MAJOR FINDINGS AND PROPOSED COURSE: A procedure has been developed for the extraction, cleanup and determination of chlorinated dibenzofurans and dibenzo-p-dioxins from soil. Final recoveries depend upon the levels of organic matter (humus) in the soil but are not less than 50% and often exceed 90%. Recovery of PCBs is variable depending upon their volatility. The importance of effective cleanup has been seen relative to quantitative interference (matrix effects) even in the absence of qualitative interferences. Extraction methods for polybrominated compounds in tissues have been improved. A rapid, semi-quantitative assay for high affinity, dioxin-binding proteins has been developed. Factors influencing the quantitative responsivity of the mass spectrometer to halogenated aromatic compounds have been identified. Future work will depend upon identified needs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The analytical methods developed as part of this project are developed in response to expressed needs of investigators at NIEHS and directly contribute to the performance of their studies. In addition, these methods relate to the goals of the Interagency Project Group for Data Quality Assurance in Analytical Chemistry.

PUBLICATIONS

Chae, K., and Albro, P.W.: Synthesis of fluorotetrachloro-dibenzo-p-dioxin. J. Environ. Sci. Health, 17B: 441-445, 1982.

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. Anal. Chem., 54: 1866-1870, 1982.

Rogan, W.J., Gladen, B.C., McKinney, J.D., and Albro, P.W.: Chromatographic evidence of polychlorinated biphenyl exposure from a spill. J. Am. Med. Assoc. 249: 1057-1058, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30015-09 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Chemical Ionization Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. Ronald Hass Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

S. Meyerson. Amoco Research Center

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study of the negative ion chemistry of compounds producing PO_3^- is ongoing.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

J. Ronald Hass Research Chemist LMB NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron capture negative ion MS/ charge reversal CID-MIKES. The collaboration with S. Meyerson of Amoco Research Center on the mass spectrometry of organophosphorus pesticides is continuing, and has been extended to other substituted aryl phosphates, and nucleotide phosphates. Ion structures have been investigated by comparison of charge-reversed spectra with the corresponding positive ion spectra.

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 provided 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

Lehman, T.A., Bursey, M.M., Harvan, D.J., Hass, J.R., Liotta, D., Waykole, L.: Comparison of collisional activation spectra of some positive ions produced both by charge reversal of negative ions and by decomposition of positive ions. Org. Mass Spectrom. 17: 607-611, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30020-12-LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transport and Metabolism of Phthalate Esters

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Phillip W. Albro Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

C.C. Pack, M.D., LTC, MC, USUHS

Gary Liss, M.D., CDC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	0.8	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The phthalate portion of diethylhexyl phthalate (DEHP) does not bind to macromolecules, but a portion of the ethylhexyl chain does (in vivo). Phthalate was found in each of 200 samples of human urine; ubiquitous exposure was confirmed. Metabolism of DEHP involves extracellular, microsomal, cytoplasmic and mitochondrial enzymes; over 24 metabolites have been identified in the rat. Rats are a very poor model for man. The enzymes that oxidize monoethylhexyl phthalate in rodents differ from those in primates both in positional and in stereo-specificity. Both the metabolites and the metabolic process itself may be involved in the biological activity of DEHP.

Principal Investigator and All Other Personnel Engaged on the Project:

Phillip W. Albro	Research Chemist	LEC	NIEHS
James R. Hass	Research Chemist	LEC	NIEHS
Kun Chae	Chemist	LEB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Isotopic tracers, chromatographic stripping, selective derivatization, gas chromatography, radio-HPLC, mass spectrometry, standard enzymology techniques, DNA isolation using hydroxyapatite, enzymatic digestion.

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 occurs more extensively in regenerating than in normal liver, and results in labeling of all four major nucleosides. Similar labeling of the DNA occurs in mouse liver *in vivo*. This phenomenon appears to involve metabolic incorporation into normal precursors rather than covalent binding. In contrast, the diethylhexyl phthalate metabolite 2-heptanone, which represents the seven carbon atoms not including C-1 from ethylhexanol, appears to bind to DNA spontaneously. This is obscured *in vivo* as 2-heptanone is extensively metabolized to CO_2. Phthalate has been found in nearly 200 samples of human urine, and no urine has been found completely free of it. This study is as yet incomplete. Diethylhexyl phthalate, after hydrolysis by nonspecific lipase to monoethylhexyl phthalate, is hydroxylated at several sites on the ethylhexyl chain by liver and kidney microsomes in the presence of NADPH *in vitro*. Oxidation beyond this point does not occur with purified microsomes. Over 24 metabolites of diethylhexyl phthalate have been identified in rat urine. Relative levels of the ten major metabolites in urine have been compared for seven animal species and wide species variability found. Different species have different absorption thresholds below which diethylhexyl phthalate is not absorbed intact. Future studies should include *in vitro* elucidation of the metabolic pathway, which involves extracellular, microsomal, supernatant and mitochondrial enzymes. The metabolite distributions should be determined for normal, diabetic, and rhenopathologic humans. The effect of chronic exposure to phthalate ester on the NAD(P)/NAD(P)H ratios in liver should be determined, as well as the biological properties of the major diethylhexyl phthalate metabolites. Similar studies should be applied to compounds other than diethylhexyl phthalate.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since diethylhexyl phthalate is a compound to which everyone in any industrilized country has a chronic, low level exposure, and since it has been shown to be associated with hepatocarcinoma in both rats and mice as well as producing testicular atrophy in male rats, the relationship between its biological activity and its metabolism in various species including man must be determined. Since the metabolic pathway is uncommonly elaborate, involving metabolic capabilities not previously anticipated, studies of the metabolic machinery, in rat

liver cells especially, should spotlight processes applicable to a wide variety of xenobiotics. Environmental chemicals whose undesirable biological effects are mediated by their metabolites or by the process of metabolism itself do not fit easily into current short-term assays for hazard potential unless their metabolism can be accomplished using a simple post mitochondrial supernate or bacteria. The metabolism of phthalates is entirely different in bacteria from in mammals, and is merely begun by the enzymes present in a post mitochondrial supernatant. Studies using these compounds as models will generally increase our ability to both predict and test for health hazards associated with environmental chemicals having highly complex catabolic pathways.

PUBLICATIONS

Albro, P.W., Corbett, J.T., Schroader, J.L., Jordan, S., and Matthews, H.B.: Pharmacokinetics, interactions with macromolecules, and species differences in metabolism of DEHP. Environ. Health Persp. 45: 19-25, 1982.

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. 45: 11-17, 1982.

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. 244: 65-79, 1982.

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 vivi. Chem.-Biol. Interact., 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. J. Environ. Sci. Health, B17; 701-714, 1982.

Sherman, L., Thompson, K., O'Kell, R.T., Albro, P., and Inkster, M.: Phthalate levels in microwave thawed fresh frozen plasma. Transfusion, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30034-08 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry of Aromatic Compounds and Their Environmental Transformation Products

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Louis A. Levy Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

The development of rational synthetic routes to polynuclear aromatic hydrocarbons and their metabolites have been investigated. Models appropriate to the study of the chemical, physical and spectroscopic properties of these classes of compounds as potential persistent environmental agents have been prepared.

Principal Investigator and All Other Personnel Engaged on the Project:

Louis A. Levy	Research Chemist	LMB	NIEHS
A.R.K. Murthy	Visiting Fellow	LMB	NIEHS

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 benzanthracenes has been extented into a new synthesis of 5-substituted chrysenes, the most biologically active of this class of polynuclear aromatic hydrocarbons. The stereochemical phenomena associated with some of the complex benzanthracenes have provided information about their conformations and the possibility of obtaining pure enantiomers from a racemic mixture of polynuclear aromatic hydrocarbons. Benzanthracenes with a functional group at C-12 have been prepared and the opportunity provided by the presence of this reactive moiety has been exploited to synthesize a number of benzanthracenes substituted at C-12 with oxygenated and alkylated groups. Some known and hypothetical oxygenated metabolites of substituted benzanthracenes have been synthesized. Several unknown or extremely rare nitrogen substituted benzanthracenes and benzanthracene derivatives have been prepared.

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.

PUBLICATIONS

Levy, L.A. and Kumar, S.: Synthesis of methyl substituted benzanthracenes and benzanthracene derivatives. Tetrahedron Letters. 24: 1217-1221, 1983.

Levy, L.A.: Synthesis of 2,3,6,7-tetrasubstituted naphthalenes; 2,3,6,7-tetrachloronaphthalene. Synthetic Comm. (in press).

Cox, R.H. and Levy, L.A.: ^{13}C - ^{13}C Spin coupling constants in tetrahydronaphthalene, hexahydrobenzanthracene and benzo(a)pyrene derivatives. Organic Magnetic Resonance. 21: 173-176, 1983.

Hass, J.R., Bobenreith, M.J. and Levy, L.A.: A study of the electron impact induced retro diels-alder process with selected 5,6,6a,7,12,12a hexahydrobenzanthracenes. Org. Mass Spect. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30050-07 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical and Enzymatic Conjugation of Glutathione with Epoxides

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Oscar Hernandez Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

Marine Pharmacology Section, LP, NIEHS and TRTP.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Procedures for the synthesis of thioether metabolites of various epoxides have been modified to allow large scale preparations. The stereochemistry of the reaction of glutathione with styrene oxide catalyzed by purified rat liver glutathione transferases has been established, and an active site geometry has been proposed to account for the stereoselectivity observed in enzyme mediated reactions.

Principal Investigator and All Other Personnel Engaged on the Project:

Oscar Hernandez	Senior Staff Fellow	LMB	NIEHS
Jack Bend	Research Pharmacologist	LP	NIEHS
M.B. Gopinathan	Visiting Fellow	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy; high pressure liquid chromatography; organic synthesis, in vitro biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: The reaction of N-acetylcysteine methyl ester with epoxides in methanol solution provides a useful, preparative scale, procedure for synthesis of mercapturic acids of alkene and arene oxides. By this approach, diastereomerically pure thioethers of styrene oxide, α-methylstyrene oxide, β-methylstyrene oxide, 1,2,3,4-tetrahydro-naphthalene 1,2-oxide, naphthalene 1,2-oxide, phenanthrene 9,10-oxide, and pyrene 4,5-oxide have been prepared.

The stereochemistry of the enzyme catalyzed reaction of glutathione with styrene oxide has been explored with purified rat liver transferases. The regiochemistry and enantioselectivity were found to be dependent on the enzyme and enantiomer used as well as substrate concentration. The finding that the enantiomers of styrene oxide differ in mutagenicity towards Salmonella typhimurium TA100 stresses the relevance of stereochemical factors in toxication-detoxication mechanisms.

The information available on the enzymatic reaction of glutathione with benzo[a]pyrene 4,5-oxide and styrene oxide has suggested a possible catalytic arrangement for the active site of the enzyme(s). The epoxide substrate is bound in a hydrophobic pocket, while glutathione is held in proximity and attack occurs from only one direction. This geometry is thought responsible for the high stereoselectivity observed in this transformation.

Proposed course includes elaboration of a synthetic scheme for glutathione conjugates similar in approach to the one developed for N-acetylcysteine derivatives, and establish the stereochemistry of conjugation for various arene oxides in order to validate the proposed active site geometry for glutathione transferases.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanistic aspects of the glutathione transferase reaction underscore the relevance of stereochemical factors in influencing the rate of elimination of enantiomeric oxides via the glutathione pathway.

PUBLICATIONS

Pagano, D.A., Yagen, B., Hernandez, O., Bend, J.R. and Zeiger, E.: Mutagenicity of (R)- and (S)-styrene 7,8-oxide enantiomers and the intermediary mercapturic acid metabolites formed from styrene 7,8-oxide. Environ. Mutagen., 4: 575-584, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30051-07 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Specific Binding Toxic Polyhalogenated Aromatic Hydrocarbons

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James D. McKinney Supervisory Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch TRTP NIEHS

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The guinea pig was used as an extremely sensitive animal model to investigate structure-toxicity relationships in polyhalogenated aromatic hydrocarbons. Results differ from structure activity relationships associated with the dioxin receptor. A protein binding model is being developed which we hope will show a better correlation with the observed structure-toxicity results.

Principal Investigator and All Other Personnel Engaged on the Project:

James D. McKinney	Supervisory Research Chemist	LMB	NIEHS
P.W. Albro	Research Chemist	LMB	NIEHS
Kun Chae	Research Chemist	LMB	NIEHS
E.E. McConnell	Veterinary Pathologist	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic methods along with X-ray crystallography and other methods for measuring physical/chemical properties with associated equipment and techniques were used primarily in this phase of the work. Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy, using specifically labeled (^{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. Computational methods are also being used to aid our ability to analyze, interpret and rationalize experimental observations and permit the ability to predict the potential of other compounds to behave similarly.

MAJOR FINDINGS AND PROPOSED COURSE: The guinea pig was used as an extremely sensitive animal model to investigate structure-toxicity relationships in poly-halogenated aromatic hydrocarbons. Lethality in guinea pigs appears to be associated with toxic planar (or coplanar) compounds with potentiation possible by certain favorably substituted halogenated biphenyls. This is consistent with quantum chemical calculations which aid in estimating populations of coplanar conformers in the halogenated biphenyls. This differs from the structure-activity relationship associated with the dioxin receptor. We seek a model based on polarizabilities that will account for the observed differences.

A protein binding model is being developed which we hope will show a better correlation with the observed structure-toxicity results. The exact nature of this binding is being studied using X-ray crystallographic, molecular graphic and theoretical chemistry approaches. Other work will attempt to show the relationship of such binding to the mechanism of dioxin and related compound 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., Gottschalk, K.E. and Pedersen, L. A Theoretical Investigation of the Conformation of Polychlorinated Biphenyls. J. Mol. Struct. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30064-06 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mass Spectrometry Studies on Low Volatility Samples

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. Ronald Hass Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The optics of a double-focusing mass spectrometer were upgraded to increase mass range and scan rate. Focusing optics were added to a secondary ion mass spectrometer source. A focused liquid metal ion source was designed. A number of compounds have been studied by particle beam ionization techniques.

Principal Investigator and All Other Personnel Engaged on the Project:

J. Ronald Hass	Research Chemist	LMB	NIEHS
R. Stoll	Visiting Fellow	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Particle beam ionization mass spectrometry (fast atom bombardment, secondary ion mass spectrometry, focused liquid metal ion source); linked B/E scans.

The VG 7070 F mass spectrometer was upgraded by replacement of the magnet, flight tube and collector housing to bring the instrument to the 70 E specification. This increases the mass range by a factor of four (to 2800 daltons at 4000 V beam energy) and the maximum scan rate by a factor of seven to 0.1 seconds/decade (in mass). The fast atom bombardment source on this instrument is now in routine use in support of IRP research projects.

A secondary ion mass spectrometry (SIMS) type source has been constructed for the ZAB/2F spectrometer. This source was found to give comparable performance to the commercial version. The incorporation of elementary focusing optics on the primary ion beam permitted a reduction of beam intensity by a factor of ten for the same secondary ion yields. As a consequence, sample depletion was similarily reduced. The overall improvement in sensitivity for polypeptides was ca. ten-fold.

Particle beam ionization techniques have been applied to a number of biological molecules. Bombesin and related polypeptides are readily analyzed by means of these techniques. The structure of the sulfate addition products of aflatoxins were determined with the assistance of fast atom bombardment (FAB). The FAB technique was found to permit the measurement of the mass spectrum of polynucleotides with as many as four nucleic acids. By combination with MS/MS techniques, the information relating to sequence could be obtained. We are currently developing a number of collaborative projects to apply particle beam techniques to specific research problems within the IRP.

A focused primary ion source with potential beam diameter of 0.5 micron or less has been designed and is under construction. After evaluation and any indicated development, this source will be used to begin a new project in microchemical morphology in environmental/biological specimens.

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.

PUBLICATIONS

Walther, H.J., Parker, C.E., Harvan, D.J., Voyksner, R.D., Hernandez, O., Hagler, W.M., Hamilton, P.B., and Hass, J.R.: Analysis of aflatoxins and reaction products of sodium bisulfite with aflatoxins by fast atom bombardment mass spectrometry. J. Agr. Food Chem. 31: 168-171, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30065-06 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Mass Spectral Reactions in Field-Free Regions

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. Ronald Hass Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.4	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tandem mass spectrometric (TMS) techniques have been applied to a variety of environmental and biomedical problems. It was found that TMS gave more rapid analyses but at lower specificity than GC/MS. Kinetic energy release measurements were found useful for structural analysis. The charge reversal process was demonstrated to give reliable results for ion structure determinations.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

J. Ronald Hass	Research Chemist	LMB	NIEHS
C.E. Parker	Chemist	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: Mass- analyzed ion kinetic energy mass spectrometry (MIKES); multiple quadrupole tandem mass spectrometry (TMS); combination of chromatographic techniques with above.

The hypothesis that TMS techniques could be used instead of more time GC/MS techniques for the analysis of polychlorinated biphenyls and polychlorinated dibenzofurans in environmental matrices was tested. It was confirmed that TMS techniques do permit faster sample turn-around. However, the analyses were more likely to suffer interference and the increased sample throughput was somewhat mitigated by the increased source maintenance necessary.

The utility of GC/MIKES for trace environmental analysis was extended by including kinetic energy release (KER) measurements. It was found that minimum detectable quantities (for KER measurement) were typically 10-9 g or less. The KER allows one to distinguish between halogens attached to aliphatic carbons and those attached to aromatic carbons.

Techniques previously developed for analysis for tetrachlorodibenzo-p-dioxins (exact mass measurements/high resolution GC and CID-MIKES) were applied to an environmental sample from Times Beach, Mo. As well as confirming the presence of tetrachlorodibenzo-p-dioxins, the technique revealed a large number of tetrachloro-methoxy PCBs, which could be misinterpreted as tetrachloro dibenzo-p-dioxins, with less selective methods.

These same techniques were employed in a collaborative project with LPFT, to identify some metabolites of 2-aminofluorene in an enzyme system. The hypothesis that fragmentation reactions resulting from the charge reversal of negative ions could be compared with the collisional activation spectra of positive ions for gaseous ion structural determinations was tested. It was found that for those cases in which ion structures could be demonstrated to be the same, nearly identical fragmentation patterns were observed by the two techniques. Ions known to have different structures positive and negative charges were found to give predictably distinctive spectra. The structures and fragmentation reactions of CH_3CN^+ and CH_3ONO^+ were studied by the charge reversal technique.

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 orgins. 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.

Methylnitrenium, ethylnitrenium, and dimethylnitrenium ions were prepared by charge reversal collisional activation (CR CA) of the corresponding negative ions, and their collisional activation spectra were used to support the assigned structures. MINDO/3 energies were used to evaluate relative energies of CH_4N^+ and $C_2H_6N^+$ isomers, and to examine if unstable forms rearrange spontaneously to stable ones. As in other examples, CR CA here generates forms that do not exist in an energy well, but their existence is established because fragmentation is more rapid than rearrangement to a more stable form.

PUBLICATIONS

Boyd, J.A., Harvan, D.J. and Eling, T.E.: The oxidation of 2-aminofluorene by prostaglandin endoperoxide synthetase. J. Biol. Chem. (in press).

Voyksner, R.D., Sovocool, G.W., Bursey, M.M. and Hass, J.R.: The comparison of GC/MS with MS/MS in the analysis of polychlorinated biphenyls and tetrachlorodibenzofuran. Anal. Chem. (in press).

Hass, J.R., Tondeur, Y., and Voyksner, R.D.: The use of kinetic release to differentiate between brominated compounds. Anal. Chem. (in press).

Voyksner, R.D., Hass, J.R., and Bursey, M.M.: Correlation of kinetic energy release with the structure of selected chlorinated compounds in mass-analyzed ion kinetic energy spectometry. Anal. Chem. (in press).

Lehman, T.A., Bursey, M.M., Harvan, D.J., Hass, J.R., Liotta, D., and Waykole, L.: Comparison of collisional activation spectra of some positive ions produced both by charge reversal of negative ions and by decomposition of positive ions. Org. Mass Spect. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30066-07 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Synthetic Methods for Polyhalogenated Aromatics

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James D. McKinney Supervisory Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

Bio-Organic Chemistry

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Selected halogenated biphenyls are being synthesized as models for structure-activity study. Labeled compounds are occasionally required to aid in structural characterization and identification work.

Principal Investigator and All Other Personnel Engaged on the Project:

James D. McKinney Supervisory Research Chemist LMB NIEHS

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 models for structure-activity studies in the halogenated aromatic hydrocarbon series. Two hexachlorobiphenyl, isomers, viz, 2,3,5,3',4',5'- and 2,3,4,5,3',4'-, have been prepared as models for "non inducing" and "mixed inducing" type PCB isomers respectively. A ^{13}C- labeled hexabromonaphthalene mixture has been prepared to aid in structural identification of these compounds as contaminants of fire retardent chemicals 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 or serve as research models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-05 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Chemically induced Photosensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Colin F. Chignell Chief, LMB LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to determine the role played by light-induced free radicals and active oxygen species (superoxide, singlet oxygen) in chemically induced skin photosensitization.

Irradiation of the following chemical agents in aqueous or organic solvents has been shown to produce a variety of free radicals and/or oxygen derived species: benoxaprofen, benzoxazole, 2-methylbenzoxazole, 2-phenylbenzoxazole, musk ambrette, anthracene and fluoranthene. These photoinduced species may play an important role in the phototoxic and photoallergic properties of these agents.

Principal Investigator and All Other Personnel Engaged on the Project:

Colin F. Chignell	Chief, LMB	LMB	NIEHS
Ann G. Motten	Staff Fellow	LMB	NIEHS
Krysztof Reszka	Visiting Fellow	LMB	NIEHS

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 phototoxicity 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 chemicals. The structures of the trapped radicals, and the initiating free radicals have been determined with the aid of electron spin resonance. Active oxygen species, eg. singlet oxygen and superoxide, have also been detected.

MAJOR FINDINGS AND PROPOSED COURSE:

Benoxaprofen: Benoxaprofen [2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid] is an anti-inflammatory drug that causes acute phototoxicity in many patients. The photochemistry of benzoxazole and a series of structurally related analogs has been studied in aqueous and organic solvents. When benzoxazole or 2-methylbenzoxazole were irradiated in aqueous solution they underwent photo-ionization and photohomolysis. In the presence of oxygen, the superoxide radical was detected.

Irradiation of anerobic ethanolic solutions of benoxaprofen or 2-phenylbenzoxazole resulted in hydrogen abstraction from the solvent to yield hydroxyethyl and ethoxyl radicals. In the presence of suitable electron donors eg. NaN_3, NaOH these compounds acted as "shuttles" and transferred the abstracted electron to an appropriate acceptor. When oxygen was present, electronic energy transfer took place to give singlet oxygen, a powerful oxidizing agent.

These findings show that benzoxazoles are photochemically active and that they are able to participate in electron/hydrogen transfer and/or energy transfer processes depending on the type of available substrate and the presence of oxygen.

The importance of both oxygen-dependent and oxygen-independent processes was also demonstrated in studies of the light-induced benoxaprofen-dependent hemolysis of human erythrocytes. In this system the presence of oxygen markedly increased the rate of photohemolysis. Inhibition by BHA provided evidence for the participation of free radicals in the reaction. Furthermore, irradiation of mast cells in the presence of benoxaprofen caused degranulation and the release of histamine. This may explain the urticarial response to benoxaprofen and light seen in human subjects.

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 a known cutaneous photosensitizer. Irradiation of musk ambrette in methanol with near UV light produced two radical species which exist in slow equilibrium with each other. Both are nitro anion radicals; one has a planar nitro anion group while 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 planar nitro anion radicals may be responsible for the cutaneous photosensitization reactions of musk ambrette. Musk ketone and musk xylene have not been reported to produce photosensitization or cross-react in musk-ambrette-sensitized patients. These studies are now complete and no further work is contemplated with these compounds.

Furosemide: Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) is a diuretic which is a known cutaneous photosensitizer. In ethanol solution, furosemide produced superoxide in the presence of oxygen and abstracted an electron or hydrogen atom from ethanol to form the hydroxyethyl radical in deaerated solution. In aqueous solution a carbon-centered radical was trapped in the absence of oxygen. Studies are continuing using the model compounds 4-chloro-5-sulfamoylanthranilic acid and furfurylamine to determine which structural features of furosemide are responsible for superoxide and carbon-centered radical formation.

Anthracene: Anthracene, a component of coal tar, is a potent photosensitizing agent both in vivo and in vitro. Studies in mice have shown that anthracene is photocarcinogenic. Anthracene causes a concentration dependent photohemolysis of human erythrocytes that is markedly enhanced by oxygen. Photohemolysis is accompanied by a decrease in the α-helical content of the red cell membranes and extensive crosslinking of spectrin and other membrane proteins. Singlet oxygen has been implicated in this phenomenon.

Fluoranthene: Fluoranthene is a polycyclic aromatic hydrocarbon found in coal tar. Although not carcinogenic, fluoranthene is known to be a co-carcinogen. In addition this compound is a potent photosensitizer when applied topically to guinea pig skin. Our results have shown that fluoranthene causes a concentration dependent photohemolysis of human erythrocytes that is enhanced by the presence of oxygen. Photohemolysis by fluoranthene is decreased by BHA and NaN_3, but is not affected by D_2O. Further studies are in progress to determine the precise mechanism of action of fluoranthene-induced phototoxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many drugs and other environmental chemicals 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

Motten, A.G., and Chignell, C.F.: Spectroscopic studies of cutaneous photosensitizing agents III. Spin trapping of photolysis products from sulfanilamide analogs. Photochem. Photobiol., 37: 17-26, 1983.

Reszka, K., and Chignell, C.F.: Spectroscopic studies of cutaneous photosensitizing agents IV. The photolysis of benoxaprofen, an anti-inflammatory drug with phototoxic properties. Photochem. Photobiol., in press.

Sik, R.H., Paschall, C.S., and Chignell, C.F.: The phototoxic effect of benoxaprofen and its analogs on human erythrocytes and rat peritoneal mast cells. Photochem. Photobiol., in press.

Sinha, B.K., Arnold, J.T., and Chignell, C.F.: Photo-induced binding of sulfanilamide to cellular macromolecules. Photochem. Photobiol., 35: 413-418, 1982.

Sinha, B.K., and Chignell, C.F.: Binding of anthracene to cellular macromolecules in the presence of light. Photochem. Photobiol., 37: 33-37, 1983.

Ortner, M.J., and Paschall, C.S.: The use of circular dichroism spectroscopy in the study of molecular phototoxicity in erythrocyte membranes. Photobiochem. Photobiophys., 4: 79-86, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50051-05 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Free Radical Metabolism of Polycyano Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project

Ronald P. Mason	Research Chemist	LMB	NIEHS

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. This project has been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50052-05 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Binding of Semiquinone Free Radicals from Anticancer Agents to DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Birandra K. Sinha Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 binds 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.

Principal Investigator and All Other Personnel Engaged on the Project

Birandra K. Sinha	Senior Staff Fellow	LMB NIEHS
Colin F. Chignell	Chief	LMB NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: We have previously shown that chemical reduction ($NaBH_4$) of adriamycin and daunorubicin generated the 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). This project has now been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50054-05 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Free Radical Formed Microsomal Incubations Containing CCl_4 and NADPH

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pharmacology

LAB/BRANCH

Laboratory of Molecular 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Ronald P. Mason	Research Chemist	LMB	NIEHS
Colin F. Chignell	Chief	LMB	NIEHS
C.R. Wolf	Visiting Associate	LP	NIEHS
Richard M. Philpot	Research Chemist	LP	NIEHS

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 experiment 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 demonstrate 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. This project has been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50062-04 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Enzymatic Reduction of C-Nitroso Compound

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 have been investigated.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Ronald P. Mason	Research Chemist	LMB	NIEHS
Volker Fischer	Visiting Fellow	LMB	NIEHS

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.

$$AH^- + R\text{-}\overset{O\cdot}{N}H \longrightarrow AH^{\cdot} + R\text{-}\overset{H}{N}O$$

$$R\text{-}\overset{H}{N}O + O_2 \longrightarrow R\text{-}\overset{O\cdot}{N}H + O_2^{\cdot-}$$

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. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 4-Nitroquinoline-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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50063-03 LMB

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Effects of High Pressure Sodium Vapor Lamps

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Colin F. Chignell Chief LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Colin F. Chignell	Chief, LMB	LMB	NIEHS

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 cell count (males only). No difference were observed between the two groups in the swim endurance, tail flick and hotplate tests. This project has now been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50066-03 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Reactions of Free Radicals from Serotonin and Related Indoles

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Department of Chemistry, Tubingen University, West Germany

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The electron spin resonance (ESR) spectra of the free radicals formed by the autooxidation 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-imines, the one-electron oxidation products of the corresponding indoles. At alkaline pH, autoxidation of these compounds ultimately leads to precipitates 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)$^{\cdot}$ radical. The NAD(P)$^{\cdot}$ radical then reacts with oxygen to form superoxide, which ultimately reduces cytochrome c. This work has been extended to the dihydroxytryptophans, which are neurotoxins.

Principal Investigator and All Other Personnel Engaged on the Project:

Ronald P. Mason	Research Chemist	LMB	NIEHS
Hartmut Stegman	Guest Worker	LMB	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: A serotonin free radical was observed by Borg in 1964 using electron spin resonance (ESR). 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. This project has been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50069-03 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies on the Effects of 2450 MHz Microwave Radiation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Mary J. Ortner Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

Toxicology Research and Testing Program

LAB/BRANCH

Laboratory of Molecular Biophysics/Laboratory of Behaviorial & Neurological Tox.

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. No effects were noted on microtubular protein when exposed to microwave radiation up to 200 mW/g.

Principal Investigator and All Other Personnel Engaged on the Project:

Mary J. Ortner	Senior Staff Fellow	LMB	NIEHS
Michael J. Galvin	Senior Staff Fellow	LBNT	NIEHS
Richard Irwin	Chemical Manager	TRTP	NIEHS

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. Exposure to 2450-MHz (cw) microwave radiation causes inhibition of cell division in intact cells and varied in vivo biological effects in both avian and mammalian species. Because these reported effects may result from alterations in the dynamics of microtubule formation, we studied the effects of simultaneous microwave exposure (2450 MHz, cw) during each of the three critical stages of the intracellar polymerization cycle. In addition, using circular dichroism spectroscopy, we studied the effect of microwave irradiation on the secondary structure of purified tubulin polypeptides.

METHODS EMPLOYED: The effect of 2450 MHz microwave radiation on purified tubular protein from calf brains 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 microtubular protein polymerization was studied using turbidometric techniques. Using fiber optic cables, excitation light was delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a fluid-filled, temperature-controlled waveguide. Turbidity was monitored using an identical cable and transferred through appropriate filters to standard detecting, amplification and recording devices.

MAJOR FINDINGS AND PROPOSED COURSE: The baseline turbidity of microtubular protein did not change under the influence of microwave radiation (20 or 200 mW/g SAR) and irradiation had no effect on the light-scattering properties of the depolymerized protein. EGTA-induced polymerization and cold-induced depolymerization patterns were also similar for both control and microwave-irradiated samples. The circular dichroism spectrum of purified tubulin also did not appear to be influenced by microwave irradiation, indicating a lack of effect on the protein secondary structure. The data suggest that the cellular effects of microwaves are not due to changes in microtubular proteins or their rate of polymerization. This project is complete.

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., and Galvin, M.J.: The effect of 2450-MHz microwave radiation during microtubular polymerization in vitro. Radiat. Res. 93: 353-363, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50074-02 LMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Free Radical Metabolism of Mutagenic Acridines

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Birandra K. Sinha Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Molecular Biophysics

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

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Birandra K. Sinha Senior Staff Fellow LMB NIEHS

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. This project has now been completed.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50075-02 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of Hydrazines

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Birandra K. Sinha Senior Staff Fellow LMB NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 or red blood cells and was also formed enzymatically. This radical or oxygen derived species may play a role in the toxicity of this drug.

Principal Investigator and All Other Personnel Engaged on the Project:

Birandra K. Sinha	Senior Staff Fellow	LMB	NIEHS
Ann G. Motten	Staff Fellow	LMB	NIEHS

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_β^H = 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_β^H = 19.4G and a_N^N = 1.7G, which is different than that obtained with metal ions. DMPO-H adduct is also formed in the red cells from hydralazine. The formation of hydralazyl radical is also catalyzed by the enzyme horseradish peroxidase. These studies are now complete and no further work is contemplated with these compounds.

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.

PUBLICATION

Sinha, B.K.: Enzymatic Activation of Hydrazine derivatives: A spin-trapping study. J. Biol. Chem. 258: 796-801, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50077-01 LMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Free radical intermediates of antiparasitic drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Roberto Docampo	Guest Worker	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Parasitic diseases are the most widespread of all the major human diseases currently affecting about three billion people. Effective drug treatment is nonexistent for many of them, and most of the available drugs, when adequately tested, have been shown to have mutagenic, cytotoxic, and carcinogenic activities. In recent years it becomes increasingly apparent that many reactive intermediates of xenobiotics are free radicals. The formation of free radicals, including oxygen-derived radicals, may lead to extensive cellular damage. The consequences of radical-initiated reactions may be the immediate death of the cells or may be more subtle and delayed as evidenced by the development of neoplasms. The aim of this investigation is: (a) To identify free radical intermediates generated by antiparasitic drugs by direct electron spin resonance spectroscopy or by spin-trapping techniques, and (b) to investigate the ability of parasite and mammalian cells and their subcellular fractions to generate free radicals from antiparasitic compounds. This information would help make future drug development possible on a more rational basis than has been possible hitherto. In addition, the study of the mode of action of existing chemotherapeutic and chemoprophylactic agents is necessary to maximize efficacy and to minimize toxicity.

Principal Investigator and All Other Personnel Engaged on the Project:

Roberto Docampo	Guest Worker	LMB	NIEHS
Silvia N.J. Moreno	Visiting Fellow	LMB	NIEHS
Ronald P. Mason	Research Chemist	LMB	NIEHS

PROJECT DESCRIPTION

OBJECTIVES: The aim of this investigation is (a) to identify free radical intermediates generated by antiparasitic drugs and phagocytic cells in the presence of parasites by electron spin resonance spectroscopy, and (b) to investigate the ability of parasite and mammalian cells and their subcellular fractions to generate free radicals from antiparasitic compounds.

METHODS EMPLOYED: (a) Isolation of parasite and mammalian cells and their fractions using known methods improved when necessary. (b) Determination of free radical intermediates from antiparasitic compounds by direct ESR spectroscopy or by using spin-trapping agents. (c) Study of the enzymes and cofactors involved. (d) Computer simulation of the spectra obtained to characterize radicals.

MAJOR FINDINGS AND PROPOSED COURSE: Objective (a): The free radical intermediate generated by benznidazole (one of the most effective drugs used in the treatment of Chagas' disease) in the presence of mammalian tissues and the lack of formation of this radical in the presence of Trypanosoma cruzi (the agent of Chagas' disease) were studied by ESR spectroscopy (Paper 1 of this report).

The identification of the free radical intermediates generated by metronidazole and other nitroimidazole derivatives used in the treatment of trichomoniasis in the presence of Tritrichomonas foetus (one of the trichomoniasis agents) was achieved by ESR spectroscopy (Paper 2 of this report).

The identity of the free radical intermediate generated by crystal violet (a dye used in blood banks to avoid transmission of Chagas' disease by blood transfusion) in the presence different stages of Trypanosoma cruzi was determined by ESR spectroscopy (Paper 3 of this report).

The the free radical intermediates generated by human polymorphonuclear leukocytes in the presence of antibody-coated T. cruzi were by ESR spectroscopy (Paper 4 of this report).

Objective (b): The ability of Trypanosoma cruzi and rat liver subcellular fractions to generate benznidazole free radical was established (Paper 1).

The ability of T. cruzi epimastigotes and trypomastigotes to generate a carbon-centered free radical from crystal violet was determined (Paper 3).

The literature concerning free radical formation by antiparasitic drugs and phagocytic cells was reviewed (Paper 5 of this report).

A natural continuation of this work is a detailed study of the pathways involved in free radical intermediate generation by parasites and mammalian cells. Of particular interest will be the study of the cofactors involved in the reduction of antiparasitic drugs. If some cofactors (e.g. reducing agents such as cysteine, glutathione, etc) are essential for parasite reduction of drugs, there should be a number of quite distinct ways of enhancing their effects while avoiding the toxic side effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Parasitic diseases are the most widespread of all the major human diseases currently affecting about three billion people. Effective drug treatment is nonexistant for many of them, and most of the available drugs, when adequately tested, have been shown to have mutagenic, cytotoxic, and carcinogenic activities. The study of the mode of action of existing chemotherapeutic and chemoprofilactic agents is necessary to maximize efficacy and to minimize toxicity.

PUBLICATIONS

Moreno, S.N.J., Docampo, R., Mason, R.P., Leon, W., and Stoppani, A.O.M.: Different behaviors of benznidazole as free radical generator with mammalian and Trypanosoma cruzi microsomal preparations. Arch. Biochem. Biophys. 218: 585-591, 1982.

Moreno, S.N.J., Mason, R.P., Muniz, R.P.A., Cruz, F.S., and Docampo, R.: Generation of free radicals from metronidazole and other nitroimidazoles by Tritrichomonas foetus. J. Biol. Chem. 258: 4051-4054, 1983.

Docampo, R., Moreno, S.N.J., Muniz, R.P.A., Cruz, F.S., and Mason, R.P.: Light-enhanced free radical formation and trypanocidal action of gentian violet. Science, in press, 1983.

Docampo, R., Casellas, A.M., Madeira, E.D., Cardoni, R.L., Moreno, S.N.J., and Mason, R.P.: Oxygen-derived radicals from Trypanosoma cruzi-stimulated human neutrophils. FEBS Lett., in press, 1983.

Docampo, R., and Moreno, S.N.J.: Free radical intermediates in the antiparasitic action of drugs and phagocytic cells. In Pryor, W.A. (Ed.): Free Radicals in Biology, Vol. 6. Academic Press, New York, 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50078-01 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radical Anion Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Clinical Pharmacology, VA Hospital, Mpls. MN

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The anaerobic incubation of almost all nitroaromatic xenobiotics, e.g., nitrobenzene, with the microsomal, mitochondrial, or cytosolic fractions of rat liver in the presence of either NADH or NADPH, leads to a multiple-line electron spin resonance spectrum characteristic of the nitro anion free radical. We have now demonstrated nitro anion radical formation by mitochondria using endogenous cofactors. Nitro drugs do not affect mitochondrial respiration, in particular the coupling to ADP. The site of nitro reduction, as determined by inhibitors of the mitochondrial transport chain, appears to be NADH dehydrogenase and outer-membrane NAD(P)H cytochrome c reductase.

Halogen-substituted nitro compounds are radiosensitizers and are among the most toxic nitro compounds. Loss of halide by the nitroaromatic anion should form a very reactive carbon-centered free radical, which will react with cellular macromolecules. This free radical has been detectable using spin trapping. The irreversible binding of these nitro compounds to DNA, protein, etc. should be inhibited by spin traps.

Free radical formation by hepatic microsomal cytochrome P-450 reduction of gentian violet, SO_2 and O_2 has also been investigated.

Principal Investigator and All Other Personnel Engaged on the Project:

Ronald P. Mason	Research Chemist	LMB	NIEHS
Silvia N.J. Moreno	Visiting Fellow	LMB	NIEHS
Roberto Docampo	Guest Worker	LMB	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Nifurtimox and nitrofurantoin are reduced by intact rat liver mitochondria to nitro anion radicals whose autoxidation generates superoxide anion as detected by direct electron spin resonance spectroscopy and by spin trapping experiments, respectively. Although nitroreduction occurred in the presence of respiratory substrates such as β-hydroxybutyrate, malate-glutamate, succinate, or endogenous substrates, nitro anion radical formation activity was much greater on addition of exogenous reduced pyridine nucleotides. NAD(P)H generated from endogenous $NAD(P)^+$ by intramitochondrial reactions could not be used for the NAD(P)H nitroreductase reactions unless the mitochondria were solubilized by detergent. It is concluded that the nitro reductase activity of respiratory chain enzymes is far less important than that of enzyme(s) located in the outer membrane.

In the past, studies of the anion radical metabolites formed by hepatic microsomal one-electron reduction implicated one-electron donation from NADPH-cytochrome P-450 reductase. .More recently, cytochrome P-450 has been found to transfer one electron to toxic chemicals such as gentian violet, sulfur dioxide, and molecular oxygen. When gentian violet is metabolized under a nitrogen atmosphere by rat hepatic microsomes supplimented with NADPH, a single line ESR spectrum is obtained. Either CO or metyrapone inhibits radical formation by 50%, suggesting cytochrome P-450 involvement. Under an atmosphere of nitrogen, rat liver microsomal incubations containing bisulfite (aqueous sulfur dioxide) and NADPH form a free radical with a single line ESR spectrum. The results imply that cytochrome P-450 reduces bisulfite to the sulfur dioxide anion radical. Oxygen completely inhibited the formation of this radical, which is consistent with oxygen being a competitive inhibitor for the reduced heme of cytochrome P-450. We have used the spin trapping technique to demonstrate the microsomal reduction of oxygen to superoxide, which we also found to be inhibited by CO and metyrapone.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: We have taken the approach of first searching for free radical metabolites and then, on the basis of what is known about the free radical chemistry, investigating the biochemical and toxicological implications of these free radical reactions.

PUBLICATIONS

Harrelson, W.G., Jr. and Mason, R.P.: Microsomal reduction of gentian violet; Evidence for cytochrome P-450 catalyzed free radical formation. Mol. Pharmacol. 22: 239-242, 1982.

Peterson, F.J., Combs, G.F., Jr., Holtzman, J.L. and Mason, R.P.: Effects of selenium and vitamin E deficiency on nitrofurantoin toxicity in the chick. J. Nutrition, 112: 1741-1746, 1982.

Peterson, F.J., Combs, G.F., Jr., Holtzman, J.L., and Mason, R.P.: Metabolic activation of oxygen by nitrofurantoin in the young chick. Toxicol. and Appl. Pharmacol. 65: 162-169, 1982.

Mason, R.P.: In situ microsomal radicals by ESR. In Packer, L. (ed): Oxygen Radicals in Biological Systems, Methods in Enzymology, Academic Press, N.Y. (in press).

Mason, R.P. and Josephy, P.D.: Free radical mechanism of nitroreductase. In Rickert, D. (ed.): Toxicity of Nitroaromatic Compounds, Hemisphere, New York (in press).

Josephy, P.D. and Mason, R.P.: Chemical and enzymatic nitroreduction; Free radical and diamagnetic products of nitroimidazoles: In Anders, M.W. (ed.): Bioactivation of Foreign Compounds, Academic Press, New York (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50079-01 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Free Radical Metabolite Formation by Peroxidases

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Ronald P. Mason Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Free radical pathways of prostaglandin synthase are being pursued in collaboration with Dr. Eling. Electron spin resonance investigations of the prostaglandin hydroperoxidase and a model enzyme system, horseradish peroxidase, have demonstrated the enzymatic formation of the sulfur trioxide anion (SO_3^-) from (bi)-sulfite. Prostaglandin synthase forms prostaglandins from arachidonic acid only in the presence of oxygen where direct ESR cannot detect SO_3^-. This species reacts with the radical trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as evidenced by ESR studies and the complete inhibition of (bi)sulfite-dependent oxygen uptake by DMPO.

The metabolism of benzidine and several derivatives was studied using horseradish peroxidase and prostaglandin synthase. Benzidine was oxidized to a radical cation and a charge-transfer complex composed of the benzidine and its two electron (di-imine) oxidation product. The two-electron oxidation product, the di-imine, is a resonance structure of the nitrenium ion, the proposed ultimate carcinogenic metabolite of aromatic amines.

In collaboration with Prof. West fast-flow techniques are being used to detect the highly reactive phenoxyl free radical metabolites of acetaminophen and diethylstilbestrol.

Principal Investigator and All Other Personnel Engaged on the Project:

Ronald P. Mason	Research Chemist	LMB	NIEHS
Volker Fischer	Visiting Fellow	LMB	NIEHS
Paul West	Visiting Scientist	LMB	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: There is considerable evidence for the *in vivo* oxidation of acetaminophen to an arylating intermediate, N-acetyl-*p*-benzo-quinoneimine, which may bind to tissue macromolecules and cause hepatic necrosis. We have demonstrated by fast-flow electron spin resonance spectroscopy that the horseradish peroxidase/hydrogen peroxide system oxidized acetaminophen to a transient phenoxyl free radical, which is thought to be the intermediate in the oxidation of acetaminopehn to N-acetyl-*p*-benzoquinoneimine. Both an over-modulated and a high-resolution spectrum have been obtained under fast-flow conditions. A more stable derivative of acetaminophen, with methyl groups placed at the *ortho* positions of the oxygen-centered free radical, has been prepared and used to study the dependence of free radical formation on enzyme and substrate concentration.

The dimethyl derivative will be studied by UV spectroscopy to determine the stoichiometry between free radical production and the formation of the benzo-quinoneimine. Deuterated analogs of acetaminophen are being prepared for use in the fast-flow electron spin resonance system. These spectra will be employed to obtain an unambiguous computer-simulated solution to the high-resolution spectrum of acetaminophen. Ram seminal vesicles and acetaminophen under fast-flow ESR conditions can demonstrate the oxidation of acetaminophen to a phenoxyl free radical by the mammalian enzyme prostaglandin synthase. Oxidation of the dimethyl derivative of acetaminophen is thought to produce less reactive metabolites because of the steric hindrance of the methyl groups *ortho* to the oxygen where the free radical is centered. Covalent binding studies using ^{14}C-acetaminophen and dimethyl acetaminophen will be conducted to compare the reactivities of the oxidation products of these compounds. If, as proposed, the dimethyl derivative displays a decreased binding to protein, then it may be a safer analgesic then acetaminophen.

PUBLICATIONS

Kalyanaraman, B., Mason, R.P. and Sivarajah, K.: An electron spin resonance study of a novel radical cation produced during the horseradish peroxidase-catalyzed oxidation of tetramethylhydrazine. Biochem. Biophys. Res. Comm. 105: 217-224, 1982.

Kalyanaraman, B., Mason, R.P., Tainer, B. and Eling, T.E.: The free radical formed during the hydroperoxide-mediated deactivation of ram seminal vesicles is hemoprotein-derived. J. Biol. Chem. 257: 4764-4768, 1982.

Josephy, P.D., Eling, T.E. and Mason, R.P.: The horseradish peroxidase-catalyzed oxidation of 3,5,3',5'-tetramethylbenzidine: Free radical and charge-transfer complex intermediates. J. Biol. Chem. 257: 3669-3675, 1982.

Mottley, C., Mason, R.P., Chignell, C.F., Sivarajah, K. and Eling, T.E.: The formation of sulfur trioxide radical anion during the prostaglandin hydroperoxidase-catalyzed oxidation of bisulfite (hydrated sulfur dioxide). J. Biol. Chem. 257: 5050-5055, 1982.

Josephy, P.D., Mason, R.P. and Eling, T.: Cooxidation of the clinical reagent 3,5,3',5'-tetramethylbenzidine by prostaglandin synthase. Cancer Res. 42: 2567-2570, 1982.

Josephy, P.D., Mason, R.P. and Eling, T.: Chemical structure of the adducts formed by the oxidation of benzidine in the presence of phenols. Carcinogenesis 3: 1227-1230, 1982.

Mottley, C., Trice, T.B., and Mason, R.P.: Direct detection of the sulfur trioxide radical anion during the horseradish peroxidase-hydrogen peroxide oxidation of sulfite (aqueous sulfur dioxide). Mol. Pharmacol. 22: 732-737, 1982.

Kalyanaraman, B., Mottley, C., and Mason, R.P.: A direct electron spin resonance and spin-trapping investigation of peroxyl free radical formation by hematin/hydroperoxide systems. J. Biol. Chem. 258: 3855-3858, 1983.

Josephy, P.D., Eling, T.E., and Mason, R.P.: Oxidation of p-aminophenol catalyzed by horseradish peroxidase and prostaglandin synthase. Mol. Pharmacol. 23: 461-466, 1983.

Josephy, P.D., Eling, T.E., and Mason, R.P.: Co-oxidation of benzidine by prostaglandin synthase and comparison with the action of horseradish peroxidase. J. Biol. Chem., in press.

Josephy, P.D., Eling, T.E., and Mason, R.P.: An electron spin resonance study of the activation of benzidine by peroxidases. Mol. Pharmacol., in press.

Eling, T., Boyd, J., Reed, G., Mason, R.P. and Sivarajah, K.: Xenobiotic metabolism by prostaglandin endoperoxide synthetase. Drug Metabol. Rev., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-01 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Applications of mass spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. Ronald Hass Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Analytical Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ready availability of sophisticated analytical support is essential to the timely and successful completion of many biomedical research projects. This activity provides either routine service or extensive collaborative support to research projects in environmental health sciences.

Principal Investigator and All Other Personnel Engaged on the Project:

J. Ronald Hass	Research Chemist	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mass spectrometry, possibly combined with chromatography using electron impact, positive or negative chemical ionization, or fast atom bombardment ionization.

MAJOR FINDINGS AND PROPOSED COURSE: Service analyses involve the simple provision of requested data for a well defined sample. These are now being performed at the rate of approximately 120 analyses/month. The majority of these are in support of various organic synthesis projects.

In addition to research discussed in other projects, the following is a list of the major collaborative work which is either on-going or has received a substantial amount of effort during this fiscal year.

1. Identification of epidermal growth factor. (Hernandez, DiAugustine)
2. Metabolism of Santonox. (Matthews)
3. Determination of methylated nucleosides. (I.P. Lee)
4. Metabolism of DEHP. (Albro)
5. Metabolism of Leukotrienes. (Eling)
6. Metabolism of DES. (McLachlan)
7. Structure and chemistry of humic acid. (Christman, UNC)

Two additional projects are scheduled for commencement in June 1983.

1. Determination of biogenic amines. (Hong)
2. Identification of carcinogen-nucleotide adducts. (Kaufmann, UNC)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50081-01 LMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Improvements in Fluorometric Instrumentation.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert D. Hall Staff Fellow LEB NIEHS

COOPERATING UNITS *(if any)*

Department of Physics
University of Illinois, Champaign
Urbana, IL

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many environmental agents, eg. polycylcic aromatic hydrocarbons, have interesting fluorescence properties which can be used to monitor their interaction with biological molecules. The aim of the present work is to improve the utility of available fluorometers. A simple digital circuit has been designed and constructed for use in conjunction with a phase modulation fluorometer. This modification will make it possible to distinguish the intensity contribution from two fluorescent species in a given system provided that their excited state lifetimes differ sufficiently. In addition a simple procedure has been devised to adjust phase modulation fluorometers so that reproducible lifetime measurements can be determined.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project

Robert D. Hall	Staff Fellow	LMB	NIEHS
Colin F. Chignell	Chief, LMB	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Many environmental chemicals and drugs have fluorescence properties that may be useful in monitoring their interaction with biological systems. In order to enhance the utility of fluorescence spectroscopy for such investigations a program designed to improve instrumentation has been initiated. Techniques include digital electronics and instrument modification procedures.

MAJOR FINDINGS AND PROPOSED COURSE: In collaboration with Professor C.E. Gratton a simple digital circuit for phase sensitive detection has been developed which can be easily interfaced with the Laboratory's SLM-AMINCO 4800 phase and modulation lifetime fluorometer. With the new phase sensitive detector, it will be possible to distinguish the intensity contributions of each of two fluorescent species in a mixture, provided that their excited state lifetimes differ sufficiently. It is expected that the technique will be routinely applied to biological systems containing either intrinsic or extrinsic fluorophores.

Current phase and modulation fluorometry has not attained its potential precision and accuracy in characterizing excited state lifetimes of fluorophors as a result of certain elusive instrumental artifacts associated with the megahertz transducer, the Debye-Sears acoustic tank, used to modulate the fluorescence exciting light. We have demonstrated the origin and nature of a particularly troublesome anomaly, traceable to inhomogeneities in the standing-wave configuration responsible for modulation of the light. In addition, we have devised a simple test for adjusting the equipment which greatly improves the reproducibility of lifetime measurements. Application of the corrective measure will permit us to examine in greater detail the effect of microscopic environment upon the excited state of a fluorophore.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fluorescent properties of environmental chemical agents such as the polycyclic aromatic hydrocarbons provides a unique opportunity to follow the fate of these compounds in biological systems by monitoring changes in their intrinsic fluorescence. In addition many biological molecules such as proteins exhibit intrinsic fluoresence. Thus fluorescence spectroscopy is a powerful tool for studying the interaction of chemicals with biological systems at the molecular level.

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, Ph.D., Supervisory Research Chemist, LMB

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, over 4000 milk and serum samples have been analyzed for TOCl and TOBr, over 1000 milk and milk substitutes for total chlorine and bromine, and about 500 placenta samples for total chlorine and bromine. The contract is in a no cost extension phase to complete detailed report writing. The methods for TOCl and TOBr in milk and serum have been published.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Obtainment 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.

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., Supervisory Research Chemist, LMB

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 OF PROPOSED COURSE: Using the methods developed previously on the contract, a total of over 6000 milk, milk substitutes, blood serum or placenta tissue have been analyzed for PCB and DDE content. Final analyses are being completed, detailed reports prepared, and samples of unused fluids and tissues and their extracts return shipped to the Institute. Method paper for PCBs and DDE analyses of milk, milk substitutes and serum has been accepted for publication. Interpretation of the results and correlation with biological findings are in progress and will be reported elsewhere.

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 such as PCB's and their effects on infant development and health.

SRI INTERNATIONAL
Menlo Park, CA
(NIH-N01-ES-79-0006)

TITLE: Application and Development of Procedures for the Analytical Determination of Environmental Chemicals by Radioimmunoassay.

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Chozo Mitoma

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D., Research Chemist, LMB

DATE CONTRACT INITIATED: June 1, 1979

CURRENT ANNUAL LEVEL: Expired

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: The contractor was able to reproduce the radioimmunoassay procedures developed at NIEHS for chlorinated dibenzo-p-dioxins and dibenzofurans, relative to specificity and sensitivity. The contractor was not able to develop significant improvements in either the specificity or sensitivity of the assays. Approximately 100 soil and 100 milk samples, plus about 30 partially purified extracts, were submitted to the contractor and assayed for dioxins by RIA. False positives may occur with soil extracts, and better cleanup procedures are clearly needed with this matrix. False positives have not been a problem with tissues; 95% confidence of freedom from false negatives requires a large enough sample to provide 100 picograms of tetrachlorodibenzo-p-dioxin. The contract has expired and there is no current intention to renew it.

SIGNIFICANCE TO BIOMEDICAL 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.

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 sections: Molecular and Comparative Pharmacology, Cell Pharmacology and Receptor Pharmacology.

A. Molecular and Comparative Pharmacology Section (Head: 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 and 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 investigated 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 head the program at NIEHS, Dr. Lucier the program 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 storage and chemical form of the accumulated xenobiotic 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 exposure to single chemicals. Effect of water temperature on metabolic storage and excretion processes is being studied.

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 Section of Dr. J.R. Fouts.

Recent Accomplishments:

1. Dr. Bend's laboratory:

   a. Three purified glutathione transferases isolated from little skate (Raja erinacea) liver were shown to be stereospecific for the R-configured oxirane carbon of benzo(a)pyrene 4,5-oxide and benzo(a)-anthracene 5,6-oxide.
   b. Using e.s.r., no free radicals could be detected during the cytochrome P-450-dependent metabolism of p-tolualdehyde, suggesting that the p-tolualdehyde metabolite which destroys pulmonary cytochrome P-450, is not a radical.
   c. The vasculature walls of rabbit liver, heart, lung and aorta have been shown to contain cytochrome P-450 isozyme forms 2, 5 and 6 in animals treated with TCDD, using immunochemical techniques. We could not identify form 4 in heart, lung or aorta.

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 polycyclic aromatic hydrocarbons (PAH) results in the induction of the synthesis of at least two cytochrome P-450 isozymes, forms 4 and 6, and in the repression of the synthesis of at least two others, forms 2 and 5. In contrast, only one isozyme (form 6) is induced in the lung by PAH and there is little or no repression of isozymes 2 and 5.

b. A direct immunochemical quantitation assay has been developed that eliminates the requirement for electrophoresis in the "Western blotting" procedure.
c. Cytochrome P-450, isozyme 6, has been detected in lungs of some untreated rabbits and the presence of this enzyme is expected to be quantitatively important in the metabolism of carcinogenic PAH, such as benzo(a)pyrene.

3. Dr. Pritchard's laboratory:

a. The proton-dependent basic amino acid transport system localized on the luminal membrane of the renal tubule has been characterized with regard to specificity and driving forces.
b. The teleost renal sulfate transport system has been shown to be driven by a pH gradient-dependent mechanism at the basolateral membrane and luminal sulfate exit to be mediated by anion exchange (for HCO_3 or Cl). In the rat, luminal uptake is mediated by Na^+/sulfate cotransport and basolateral membrane sulfate exit is mediated by exchange for HCO_3 or OH.
c. The sulfate conjugate of 4-methylumbelliferone was transported twice as effectively as its glucuronide conjugate by isolated flounder tubules in vitro. This transport was energy-dependent and blocked by probenecid in both cases.

4. Dr. Anderson's laboratory:

a. BP-metabolite-DNA adducts were quantitated in lung, liver, forestomach, brain, colon, kidney and muscle of A/HeJ mice after single oral doses (0.06 or 6 mg/mouse) of ^{3}H-benzo(a)pyrene. The specific activities of the BP-metabolite-DNA adducts did not vary more than 2-fold between these tissues and the HPLC profiles of metabolite-deoxynucleosides were qualitatively very similar in each case. In contrast, there was more than a 20-fold variation between the tissues in the amount of BP metabolites tightly bound to protein, suggesting that the metabolic capacity of a tissue may not be the rate-limiting step in the formation of BP-metabolite-DNA adducts.
b. Benzo(a)pyrene induced unscheduled DNA synthesis (UDS) in liver of A/HeJ mice at oral doses of 0.3 and 3.0 mg per mouse whereas UDS was not detected in lung at either dose.

B. Cell Pharmacology Section (Head: 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 elutriation and centrifugation techniques. The contribution of the metabolic systems in individual cells to target organ and cell toxicity is evalauted.

Assay systems are being developed so that both oxidation and conjugation pathways of chemical metabolism can be quantitated in single cells. Such procedures will eventually be extended to other systems, including cells in culture. The scientists in this section frequently collaborate with those in the Molecular and Comparative Pharmacology Section.

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. An antibody to NADPH-cytochrome P-450 reductase inhibited 7-ethoxycoumarin O-deethylase and p-nitroanisole O-demethylase activities 90% in microsomes of rabbit type II cells and whole lung, but only 50% in microsomes of Clara cells, consistent with the idea that there is more cytochrome b_5 involvement in the oxidative metabolism of these substrates by Clara cells than by type II cells.
   b. In contrast to rat lung, very little prostaglandin synthetase activity or arachadonic acid (AA)-dependent oxidation of benzo(a)pyrene 7,8-dihydrodiol was observed in subcellular fractions of Clara cells or alveolar type II cells isolated from rabbit lung. However, hydroperoxy-eicosatetranoic acid metabolites were detected when microsomes of Clara cells, type II cells or whole rabbit lung were incubated with AA.
   c. Epidermal and sebaceous cells were freed from skin of hairless mice by digestion with pronase and separated into cell fractions by metrizamide gradients and elutriation. Early elutriator fractions were small cells (mostly basal) while later fractions were enriched in larger, more differentiated cells. Cytochrome P-450-dependent monooxygenase activity increased with differentiation, sebaceous cells having the highest activity of all cells studied.
   d. Conjugation of umbelliferone with sulfate and glucuronide was studied in mixed skin cell populations. At low substrate concentrations and at early time sulfation predominated; subsequently, or at high substrate concentrations, glucuronidation is the major pathway.

2. Dr. Schiller's laboratory:

   a. The apoproteins of the chylomicra and very low density lipoproteins (VLDL) were analyzed from the pooled mesenteric lymph fractions from a control rat (given ^{14}C-leucine) and a TCDD-treated rat (given ^{3}H-leucine). In repeated experiments, both the apo A-I and apo A-IV of the VLDL and the apo A-I in chylomicra, the predominant apoprotein of the chylomicra, were decreased in animals treated with TCDD.
   b. Probit analysis of the TCDD-induced mortality in C57BL and DBA mice indicated LD_{50} 30-day values of 180 µg/kg and 2580 µg/kg, respectively. The parallel slopes of the probit curves suggest a similar mechanism of acute toxicity in the two mouse strains.

C. Receptor Pharmacology Section (Head: Dr. G.W. Lucier)

The research of this group is concerned with various aspects of hepatoxicity 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 are 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-sepcific toxicity including cardiovascular disease, hypertension and hepatotoxicity.

Recent Accomplishments:

1. Dr. Lucier's laboratory:

   a. The female rat liver appears to be more responsive to estrogen exposure than the male liver. A dose of 20-30 μg estradiol/kg/day produces a 3- to 4-fold increase in VLDL fraction triglycerides in females, a response that requires 100 μg/kg/day in males.
   b. Concentrations of radiolabeled cytosolic and nuclear receptors were greater in isolated perfused livers of female rats than in those of male rats at all perfusion periods examined at a ^{3}H-17β-estradiol concentration of 4 nM.
   c. Zearalanol (P-1496), a derivative of the mycotoxin zearalenone, binds to the rat hepatic estrogen receptor as well as estradiol and treatment of rats with P-1496 produces selective changes in hepatic protein synthesis.
   d. There are cytosolic binding proteins in liver cytosol of male rats that have high affinity for androgens. These androgen binding sites translocate androgens to the nucleus, bind to DNA, and their presence correlates with several androgen-responsive functions in rat liver, suggesting that these high affinity androgen-binding proteins behave as an androgen receptor.

2. Dr. Fowler's laboratory:

   a. The previously reported 11.5 and 63K dalton Pb-binding components of rat kidney cytosol are saturable and possess a high affinity for Pb^{2+}. Moreover, the binding capacity of these proteins in altered (often increased) by the presence of other metal ions and high concentrations of lead.
   b. In vivo experiments demonstrated that the nephrotoxicity caused by cadmium metallothionein occurs as a result of intracellular Cd^{2+} release following degradation of Cd-metallothionein in proximal tubule cell lysosomes.

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.

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 with Drs. Mike Meredith and Fred Guengerich, Vanderbilt University; Dr. Fouts with Dr. Ping Pan, U.S. Department of the Interior, and Dr. Leakey at the University of Dundee; Dr. Lucier with Dr. Kern of the University of Colorado Medical Center, and Dr. Mary Vore, University of Kentucky; Dr. Philpot with Dr. Eric Johnson of Scripps Clinic and Research Foundation, Dr. Charles Plopper, University of California Davis, and Dr. Ed Bresnick, University of Vermont; Dr. Pritchard with Dr. David Miller, Michigan Cancer Center, Detroit, and Dr. Arnost Kleinzeller, University of Pennsylvania; Dr. Anderson with Dr. Bob Dedrick, Chemical Engineering Section, NIH; 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. J.P. Bogaert, Department of Toxicology, Rhône-Poulenc, Paris, France.

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'83 were Dr. Masahiko Negishi (Visiting Scientist with Dr. Bend), Dr. Yukio Kato (Visiting Associate with Dr. Negishi), Dr. James Mathews (Staff Fellow with Dr. Bend), Ms. Destiny Brier-Russell (GS-9 Technician with Dr. Bend), Mr. Peter Bent (GS-9 Technician with Dr. Philpot), Dr. Ellen Cheung (Visiting Fellow with Dr. Bend), Dr. Nobuhiro Harada (Visiting Fellow with Dr. Negishi), Dr. Julie Horton (Visiting Fellow with Dr. Bend), Dr. Thomas Massey (Visiting Fellow with Dr. Fouts), Dr. Zahra Parandoosh (Visiting Fellow with Dr. Philpot), Dr. Felix Romagna (Visiting Fellow with Dr. Anderson), and Dr. Joseph Rosenior (Visiting Fellow with Dr. Anderson). In addition, we had several post-doctoral guest workers in the Laboratory of Pharmacology this year. These included Dr. Jean-Paul Bogaert (with Dr. Schiller), Dr. Stephen Belinsky (with Dr. Anderson), Dr. Lori Dostal (with Dr. Bend), Dr. Allison Vickers (with Dr. Lucier), and Dr. Betty Warren (with Dr. Philpot). Dr. Ivan Gut and Dr. Wolfgang Klinger (Dr. Fouts) and Dr. Regina Brigelius (Dr. Bend) also spent several months with us as Visiting Scientists or Associates. Unfortunately for LP, we lost two employees who had been with us for many years, and who also were very productive and dedicated (Mr. Gary Foureman in Dr. Bend's laboratory since 1972 and Ms. Bobbi Pohl who came to NIEHS with Dr. Fouts in June, 1970).

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 Board of Editors, Environmental Health Perspectives; Visiting Scientist, C.V. Whitney Marine Laboratory, University of Florida, St. Augustine; member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions; Associate

Invited to present seminars at the School of Public Health, University of Washington and Rockefeller University; Selected as a member of the Scientific Committee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health; ASPET representative to the organizing committee for the 1984 FASEB meeting for theme on Repair of Tissue Injury.

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 participant at International Symposia on Extrahepatic Drug Metabolism and Chemical Carcinogenesis, May 17-20, Stockholm, Sweden; and on Metabolism and Chemical Carcinogens, May 24, Oslo, Norway; Presented a research seminar to the Departments of Biochemistry and Pharmacology, University of Ann Arbor, Michigan.

Dr. J.B. Pritchard: Adjunct Associate Professor, Department of Pharmacology, University of Florida School of Medicine, Gainesville; Invited participant, Symposium on Renal Transport Mechanisms, Satellite Symposium of the International Physiology Congress, Sydney, Australia; Invited speaker at an EPA Symposium on Reducing Hazards to Aquatic Organisms.

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 Fellows in Toxicology Training Program.

Editor, Reviews in Biochemical Toxicology; Coordinator for "Special Topics in Toxicology" a graduate course given 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 Symposia on "Extrahepatic Drug Metabolism and Chemical Carcinogenesis," May 17-20, Stockholm, Sweden; on "Metabolism and Chemical Carcinogens," May 24, Oslo, Norway; on "Responses of Marine Organisms to Pollutants," April 27-29, Woods Hole; on "Perspectives in Toxicology in Ontario," May 5-6, London, Ontario; and on "Lung Toxicity: Mechanisms and Biological Consequences," June 3-4, Rutgers, New Jersey; presented research seminars at the University of Toronto and the University of Wisconsin, Milwaukee; also participated in the NIH Intramural Scientific Research Seminar series in Bethesda.

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; Associate Editor, Pharmacological Reviews; Editorial Board of Xenobiotica, Pharmacology, Journal of Toxicology and Environmental Health; Lectures to graduate students at UNC - e.g., - three lectures in Pharmacology 206, Biotransformation of Xenobiotics and discussion panels; served on graduate committees - e.g., - Lori Dostal, Department of Pharmacology, UNC.

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 Pediatric Pharmacology and Journal of Applied Biochemistry; Invited speaker to International Conference on Prevention of Physical and Mental Defects, Sex Differentiation of Liver Protein Synthesis; Serono Symposium on Sexual Differentiation: Basic and Clinical Aspects, Sex Dimorphism of Hepatic Estrogen and Androgen Action; University of Illinois at Chicago, Imprinting of Hepatic Enzymes; International Conference on Toxicity of Chlorinated Biphenyls, Dibenzofurans and Dibenzodioxins, Mechanism of Action of Toxic Halogenated Aromatics; Temporary Advisor to International Agency for Research on Cancer, Mechanisms by which Hormones Influence Carcinogenesis; 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. M.W. Anderson: Adjunct Associate Professor, North Carolina State University, Biomathematics and Toxicology Department; member of the committee on Pyrene and Selected Analogs, National Research Council, National Academy of Sciences; Invited participant at Symposium on Extrahepatic Drug Metabolism and Chemical Carcinogenesis in Stockholm, Sweden and invited presentation at Symposium on Drug Metabolism and Chemical Carcinogenesis in Orlo, Norway.

Dr. B.A. Fowler: Adjunct Associate Professor, Department of Pathology and Toxicology Curriculum, University of North Carolina, Chapel Hill; member, Editorial Boards Chemico-Biological Interactions and Environmental Health Perspectives; Co-chairman and participant at the American Association of Pathologists Minisymposium on Toxic Cell Injury; Invited participant Second International Symposium on Responses of Marine Organisms to Pollutants, Woods Hole, Massachusetts; Co-Chairman, NIEHS/IPCS/WHO Conference on Metallothionein and Cadmium Nephrotoxicity; Dahlem Conference on Changing Biogeochemical Cycles of Metals and Human Health (Rapporteur

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 35005-04 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacokinetic Considerations in the Formation and Repair of Carcinogen-DNA Adducts

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Marshall W. Anderson	Mathematician	LP	NIEHS

COOPERATING UNITS *(if any)*

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:
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 *(Use standard unreduced type. Do not exceed the space provided.)*

There is compelling evidence that many mutagens and carcinogens are able to react with cellular DNA either directly or following metabolic activation to reactive metabolites. If DNA replication proceeds on such a modified template before altered bases or nucleotides are removed by enzymic repair processes, the mutations can be genetically fixed. Thus, 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 carcinogen metabolite-DNA adducts in target and non-target tissues for carcinogen-induced neoplasia, with emphasis on benzo(a)pyrene. We are concerned with the effect of dose of carcinogen and inhibitors of carcinogenesis on the amount and type of adducts formed. We are concerned with the adduct dose and time after carcinogen exposure on the excision repair of bulky adducts. Emphasis is on studies which enhance our understanding of the relationship between metabolism of carcinogen and the amounts and types of DNA adducts formed in the various tissues.

PI:	Marshall W. Anderson	Mathematician	LP NIEHS
Other:	Dr. Mahmooda Kulkarni	Senior Staff Fellow	LP NIEHS
	Ms. Jill Stowers	"Q" Appointment	LP NIEHS
	Dr. Joseph Rosenior	Visiting Fellow	LP NIEHS

PROJECT DESCRIPTION

OBJECTIVES: 1) To examine the in vivo formation and repair of carcinogen metabolite-DNA adducts in various tissues and in various species, with emphasis on the carcinogen benzo(a)pyrene.

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 carcinogen metabolite-DNA adduct formation and/or repair of such damage can explain the difference in organ and species susceptibility to carcinogen-induced neoplasia.

4) To examine the effect of the initial amount of carcinogen metabolite-DNA adducts formed on the enzymatic repair of the adducts in a target and non-target organ for carcinogen-induced neoplasia as well as the effect of adduct formation on de nova 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 examine the formation of BP metabolite-DNA adducts in specific DNA sequences such as the repeating Alu sequences and the excision repair of adducts in these sequences.

8) 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 doses of ^{3}H-BP and then sacrificed at various time points. DNA was isolated from tissue by phenolic extraction plus hydroxyapatite chromatography. Isolated DNA was enzymatically digested to individual nucleosides. A high pressure liquid chromatography (HPLC) procedure was developed to analyze for BP metabolite-deoxynucleoside adducts. A technique to label the BP metabolite-deoxynucleoside adducts with ^{32}P is being explored as an alternative way to quantitate adduct levels. This would eliminate the necessity of administering radiolabeled compounds to the animals.

2) Carcinogen-induced unscheduled DNA synthesis (UDS) was examined by separating de nova 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 doses (6 and 0.06 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.

It was also observed that the BPDEI and BPDEII adducts are formed in the brain of the rabbit and the levels in the brain are approximately 50% of those found in lung and liver. We will examine the BP metabolite-DNA adduct levels in some isolated cell types in the lung and liver of rabbit after in vivo exposure to BP. These cell types will include the hepatocytes and non-parenchymal cells of liver and type II, macrophage, and endothelial cells of lung. It is important to determine if, in general, BP metabolite-DNA adducts are being formed in most tissues and cells after low dose exposure since BP as well as other polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants.

2) We examined in vivo DNA repair synthesis in liver and lung of A/HeJ mice exposed to benzo(a)pyrene (BP) and 4-nitroquinoline-1-oxide (4-NQO). To differentiate between the removal of carcinogen metabolite-DNA adducts due to cell turnover and due to DNA repair, we measured unscheduled DNA synthesis (UDS) in the non-replicating DNA fraction. BP induced UDS in liver at oral doses of 0.3 and 3.0 mg per mouse whereas UDS was not detected in the lung. Our technique was capable of detecting UDS in lung since 4-NQO did induce UDS in lung. This is the first report of UDS in vivo following BP administration (or any polycyclic aromatic hydrocarbon). These and previous results clearly demonstrate that in vivo disappearance of adducts cannot necessarily be equated with nucleotide excision repair.

The presence of excision repair in the liver and its low rate of DNA synthesis provide an explanation for the relative resistance of this tissue to carcinogenesis by BP, since, under altered conditions of DNA replication following hepatectomy, tumors can be induced by PAH treatment. In contrast to the liver, the lack of excision repair in vivo of BP metabolite-DNA adducts and the relatively high rate of DNA turnover in lung are favorable conditions for the fixation of promutagenic lesions. We will continue to examine UDS as a function of carcinogen dose and time after exposure to carcinogen in various tissues (see No. 1 of this section) of various species and strains of mice. We will examine UDS in specific DNA sequences such as the repeating Alu sequences. We will also develop an in vivo - in vitro approach for measuring UDS.

3) 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. We have

also shown that BHA treatment inhibits BP metabolite-DNA adduct formation over a large BP dose range, 2-to-1350 umol/kg. These results are consistent with the hypothesis that BHA inhibits BPDE adduct formation by altering the oxidation of BP-7,8-diol. A project has been initiated with Drs. Wattenberg and Lam of the University of Minnesota to compare the anticarcinogenic effects of BHA and kahweol palmitates. These chemicals appear to behave similar as anticarcinogenic agents, but kahweol is more potent. Kahweol is a natural ingredient of coffee beans.

4) 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 isozymes 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 induers inhibit the in vivo formation of BPDE-DNA adducts can be explored more readily in rabbits than in mice. These studies are being done in collaboration with Drs. J.R. Bend and R.M. Philpot.

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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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70132-04 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intestinal Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

C. M. Schiller Research Chemist LP NIEHS

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Pharmacology

SECTION

Cell Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina; UNC, Chapel Hill, N.C.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 the alteration of these normally occurring events in response to oral exposure to biologically active environmental toxins. Currently, our investigations involve the systematic examination of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) on normal lipid assimilation. The mechanism of the physiologic changes is monitored with a combination of *in vitro* and *in vivo* techniques. In particular, our studies include examination of 1) chylomicron formation, transport and metabolism, 2) apoprotein changes in lymph lipoproteins (chylomicra and very low density lipoproteins), 3) dose-related responses to treatment and 4) genetic component of metabolic response.

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PROJECT DESCRIPTION

METHODS EMPLOYED: Our experimental protocol to produce the TCDD-induced lesions in rats is based on an initial observation by E.E. McConnell (NIEHS). Animals are weighed and fasted overnight. Generally, we use adult, male Fischer rats (10 weeks old). The next day the animals are assigned randomly to either a control or treated group. The treated animals are given 1 ml corn oil/250 g body weight by gavage. The oil contains TCDD at the level of 80 ug/kg body weight. The control animals are given 1 ml corn oil/250 g body weight by gavage. One week after the initial weighing, the animals are weighed again and fasted overnight. The next day the animals are used in a variety of experiments. When lipid assimilation is examined, the animals are given 1.5 ml corn oil by gavage.

In order to examine the lymph lipoproteins, it was necessary to develop the mesentric lymph cannulation procedure in our laboratory. The well-established procedure of Bollman et al. is followed. The animals are given corn oil by gavage and Nembutal by i.p. injection (0.1 ml/100 g body weight). After incision, a PL-50 cannula is inserted into the mesentric lymph duct. A second cannula is inserted into the duodenum 1 cm proximal to the bile duct. The wound is closed and the rat is given an infusion mixture of 0.85% NaCl-0.03% KCl via the duodenal cannula. Depending on the experiment, either radioactive leucine is injected in the tail vein at the time of gavage or the corn oil is labeled with radioactive lipid precursor. The lymph is then collected at 4°C in hourly fractions.

An additional microsurgical technique was necessary to perform lipoprotein clearance studies. The lymph chylomicra were isolated from control and treated animals and reinjected into a second group of control and treated animals. In the second group, the animals were lightly anethetized with ether before exposure of the right jugulur vein. Aliquots of radiolabeled chylomicra were injected quickly and the wound closed. Blood samples were then taken over a time course or blood and tissues were taken at particular times.

Analysis of the apoprotein synthesis in control and treated animal intestine after fat feeding required us to develop lipoprotein isolation and protein purification techniques. The radiolabeled lymph collection was pooled and centrifuged to spin up the chylomicra and VLDL fractions. Extraneous (serum) proteins were removed from these lipoprotein fractions by passage through an agarose column, Bio-Gel A-50m, and the lipoproteins were collected for use in the void volume only. The void volume fractions were pooled and, then concentrated and dialyzed against an Amicon YM-10 membrane. The concentrated, salt-free lipoprotein fractions were lyophilized and delipidated with diethylether. The remaining apoprotein was solubilized in 1% SDS-5% β-mercaptoethanol before polyacrylamide disc gel electrophoresis. The gels were stained for protein with Coomassie blue and scanned before slicing (2 mm slices), digesting and counting.

Comparative studies were performed in another species, mice, which is known to have a TCDD-resistant strain (DBA/2S) and a TCDD-sensitive strain (C57BL/6J). The rat protocol was adapted for mouse use by determining the LD_{50}-30 for each strain of mice and then examining lesions one week after treatment with equitoxic doses of TCDD. In addition, it was necessary to adapt the in vitro uptake techniques previously used with rats for mice, e.g., obtaining adequate serum volumes for analysis and revising in vitro gut sac uptake techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Model Protocol: Our experimental model described above is well-established and is generally used throughout our investigations. Based on this model, we have further characterized the syndrome associated with a single oral exposure to TCDD. Weight loss occurs within one week after treatment, with as little as one-sixth of the LD_{50}-30, i.e., 10% (net body weight loss). Several serum parameters change one week after treatment: 1) glucose levels decrease to as much as one-half of control values; 2) triglyceride levels increase by 2-3 times the control values; and 3) both free and esterified cholesterol levels increase by a factor of two. In contrast, serum protein (total) levels are similar and serum insulin levels are the same. Both serum glucose and insulin levels exhibit a normal response with time during a glucose tolerance test. Since high insulin levels did not drive glucose from the serum into insulin-sensitive cells in TCDD-treated animals, the possibility of altered active glucose transport in an insulin insensitive tissue was examined. The use of intestinal tissue fulfilled this requirement of insulin insensitivity, and also permitted an evaluation of nutrient absorption from dietary sources. Both amino acids and monosaccharides are actively transported by intestinal epithelial cells.

As measured by an in vitro gut ring technique, active transport of amino acid and monosaccharide are not changed substantially by TCDD treatment. Based on morphological examinations of intestinal epithelium after fat feeding, it appears that lipid droplets aggregate abnormally in treated animals. Time course experiments with [1-^{14}C]-triolein labeled corn oil revealed that, in fact, lipid is absorbed and does appear in the rat serum after fat feeding. The pattern of appearance of label in the serum suggests increased absorption and/or decreased clearance of lipid from the serum in the treated animals. At the peak of lipid absorption, the serum levels rise as much as 6-fold higher in the treated animals as compared to the control levels.

Of particular interest is that this increment in serum lipid levels in treated animals is positively correlated with dose of TCDD and it does not plateau at the highest dose given (90 µg TCDD/kg body weight). Even on the ad libitum diet, without fat feeding, the serum triglycerides increase with dose. The NIH-31 diet is less than 1.5% fat by weight. These results support altered lipid transport and metabolism as a consequence of treatment. Other dose-related parameter changes were body weight and tissue weight, e.g., thymus, epididymal fat pad, and perirenal fat pad. A detailed LD_{50}-30 study indicates a value of about 420 µg TCDD/kg body weight for the adult, male Fischer rat.

Changes in Triglyceride Metabolism and Transport: In addition to changes in serum triglyceride levels in ad libitum (low fat) and in fat-fed animals, TCDD treatment alone significantly increased tissue triglyceride levels in liver and intestinal mucosa within one week after treatment, increases of 105% and 67%, respectively.

However, this tissue triglyceride increment was not readily observed by transmission electron microscopy. To determine whether TCDD treatment precluded the development of a grossly fatty liver, groups of control and treated animals were given a single oral dose of 50% ethanol (2.0 ml/250 g body weight) and then sacrificed 16 hours later. Whether the liver had an altered capacity to remove triglycerides from the serum lipoproteins after TCDD treatment has not yet been determined. This acute challenge of ethanol readily induced a fatty liver and gut within 16 hours in both the control and treated animals, for example in liver, increases of 163% and 181%, control and treated, respectively. Further investigation is required to determine if the same mechanism is involved in the control and treated animals, and if there is a greater stimulation by ethanol of hepatic lipid synthesis in the TCDD-treated animals than in the control animals.

Since increases in serum triglycerides may reflect increased absorption, altered transport and/or altered removal, we examined the levels of serum lipoprotein lipase (EC 3.1.1.3). When serum lipoprotein lipase was measured, however, the pre-heparin levels were not detectable in serum from control animals, but were readily measurable in serum from TCDD-treated animals. This lipoprotein lipase level in the pre-heparin, TCDD-treated rat serum was greater than the post-heparin lipoprotein lipase level in the control rats, only 20.9 ± 6 units/ml, and was stimulated more than 3-fold by in vivo heparin treatment. Recombination experiments with pre-heparin serum plus either one of the post-heparin sera indicate the absence of an inhibitor of the in vitro assay. The in vivo activity of serum lipoprotein lipase depends on the interaction of the endogenous substrate, chylomicron triglyceride and apo C-II at the surface of the artery wall or organ cell membrane. Thus, in vitro activity with exogenous substrate is not proof of substantial activity in vivo. Currently, we plan to examine the tissue distribution and location as well as the heparin induced release of lipoprotein lipase in control and TCDD-treated animals.

An additional approach that we are using to examine the metabolism of the dietary lipid obtained from lymph chylomicra involves 1) fat-feeding with corn oil tagged with ^{14}C-palmitic acid, 2) cannulation and collection of mesentric lymph, 3) isolation and lipid analysis of the lymph chylomicra (90% of label is in the triglyceride), and 4) injection of the labeled chylomicra into the jugular vein of control and treated rats. The examination of the appearance of labeled triglyceride in tissues with time indicates whether the lipoprotein lipase is functioning in vivo. This observation rests on the fact that tissues such as adipose tissue cannot take up triglycerides. Instead, the serum triglycerides must be converted to free fatty acids and glycerol in the serum. The free fatty acid are then taken up by the adipose tissue and used for the synthesis of tissue triglycerides by the α-glycerophosphate pathway. The T-1/2 for the clearance of the chylomicra lipid from the blood is also being determined.

Lymph Lipoproteins: The appearance of lipoproteins in the lymph after fat feeding was followed with time. The pattern of protein (total) found in the lymph of control animals parallels that of the triglyceride in the lymph, each expressed as mg/ml. In contrast, from about the 10th hourly fraction to the 16th hourly fraction, the triglyceride appearing in the treated rat lymph increases while the protein decreases. The decrease in protein in the treated lymph may represent a failure to synthesize the necessary apoproteins for chylomicron formation prior to exit from the epithelial cell. When less fat is given -- by infusing a micellar

solution instead of gavage -- the amounts of triglyceride and protein more closely parallel to each other.

The apoproteins of the chylomicra and VLDL fractions were analyzed from the pooled lymph fractions from a control rat, given ^{14}C-leucine, and a treated rat, given ^{3}H-leucine, each by the tail vein. Separation by SDS-PAGE revealed the characteristic bands apo B, apo A-IV, apo E, apo-AI and apo C proteins. The proteins were identified based on their known molecular weights and molecular weight standards run on parallel gels. The ratio, ^{3}H/^{14}C, of each gel slice was compared to the sample added initially to the gel to determine relative changes in protein synthesis. In repeated experiments, both the apo A-I and apo A-IV of the VLDL and the apo A-I in the chylomicra are decreased after TCDD-treatment. The predominant apoprotein of the chylomicra is apo A-I or about 38-50% of the total chylomicra protein. Thus, the importance of apo A-I to the surface structure of the chylomicron is obvious. Although the fate of apo A-IV is unknown, apo A-I is the binding component for lecithin:cholesterol acyltransferase and it probably follows the fate of the high density lipoprotein. Further experiments are underway which produce apo B more expenditiously since this protein tends to decay with time after isolation. Serum composition studies are being performed to verify the location of the elevated lipids observed in treated animals as to their relative concentration in the various lipoproteins.

Genetic Variant Mouse Model: The genetic component of TCDD toxicity has been previously established using the TCDD sensitive C57BL and resistant DBA strains of mice. A variety of toxic effects; cleft palate, thymic involution and microsomal enzyme induction have been used to characterize the responsiveness of these two strains of mice to TCDD exposure. Each response has been shown to occur at lower doses in the sensitive C57BL strain and to segregate genetically as a simple autosomal dominant trait. Interestingly, the acute lethal toxicity of TCDD in the DBA mouse has not been previously established. An LD_{50} 30-day study was conducted to compare the acute lethal effects of TCDD in the C57BL and DBA strains of mice. Animals were given a single oral dose of TCDD dissolved in corn oil. Body weight loss, the characteristic wasting syndrome, exhibited a dose response in both strains of mice. Maximal weight losses were between 30% and 40% in the animals dosed at the highest TCDD concentrations. Significant increases ($p < 0.05$) in liver weight per kg body weight were noted in animals of both strains that survived the 30 days post exposure. Thymus weights were decreased significantly in both strains of mice. Increases or decreases in other relative organ weights, spleen, testes, gut and epidicymal fat were less consistent, although significant differences ($p < 0.05$) were noted for each organ. The earliest recorded deaths in TCDD-treated animals was 14 days after exposure. Probit analysis of the percent mortality indicates an LD_{50} 30-day value of 181 µg/kg and 2580 µg/kg for the C57BL and DBA mice, respectively. The slopes of the two probit curves are not significantly different ($p < 0.05$) and the parellel slopes are consistent with a similar mechanism of acute toxicity in the two strains of mice. The increased potency of TCDD in the C57BL mouse, 14.3 times the DBA mouse, is consistent with literature evaluations of other toxic responses.

Previous studies of the Fischer rat indicated that a single oral dose of TCDD, at 53 µg/kg, lowered fasting serum glucose to 41% of controls one week after TCDD exposure. The relationship of this hypoglycemic response to the acute toxicity of TCDD was examined in the C57BL and DBA strains of mice, in which the acute

lethal toxicity of TCDD has been well established. Dose response experiments indicate that in the C57BL and DBA mouse, TCDD exposure lowers fasting serum glucose to 0% of control at 40 µg/kg and 1800 µg/kg TCDD exposures, respectively. Time course experiments indicate that this maximal effect (0% serum glucose) is obtained 5 days after TCDD exposure. However, because 0% serum glucose is considered an unusual response, the glucose oxidase method for measuring serum glucose was examined in treated animals. Addition of glucose solutions of known concentration to serum from TCDD-treated animals indicates that substantial inhibition of the glucose oxidase method occurs in these animals. Known inhibitors of the glucose oxidase assay include: bilirubin, reduced glutathione and uric acid. The possible involvement of those compounds in the observed inhibition of the glucose oxidase assay in TCDD-treated animals is being investigated. Analysis of serum glucose in TCDD-treated animals, using an alternative assay system, indicates that TCDD treatment does reduce fasting serum glucose levels, but to a lesser extent than previously reported. Dose response experiments in the mouse indicate a serum glucose of 71% of control at the maximum TCDD concentration tested, 200 µg/kg and 2500 µg/kg for the C57BL and DBA mice, respectively. In the Fischer rat, exposure to 81 µg/kg TCDD results in a serum glucose of 88.6% of control ($p < 0.05$).

The C57BL and DBA mouse were examined for the effects of TCDD exposure on lipid absorption. A differential response in the genetically defined TCDD sensitive C57BL and TCDD resistant DBA mouse implicates the involvement of the cytosolic receptor previously postulated to mediate other responses to TCDD exposure in the lipid malabsorption syndrome described for the Fischer rat. Equitoxic doses of TCDD, 181 µg/kg and 2580 µg/kg based on previous LD_{50} studies, were used for the C57BL and DBA mouse exposures. TCDD treatment one week prior to an intubated corn oil challenge results in a decreased absorption of radioactivity labeled triolein tracer in the corn oil, as measured by the appearance of radioactivity in the serum. The significant decrease in total lipid absorption is not accompanied by changes in kinetic input or exit parameters, or the time of maximal serum radioactivity. In addition, the accumulation of lipid droplets in the intestinal mucosal cell described by E.E. McConnel in the Fischer rat is not observed in TCDD treated mice. In vitro uptake and transfer studies using everted mouse gut sacs and well-defined, micellar solutions of monoolein, sodium taurodeoxycholic acid, oleic acid and radioactive oleic acid tracer were conducted to further define the sites of TCDD interaction which result in the observed reduction in lipid absorption. In both strains of mice, initial rates of oleic acid uptake are the same in both control and treated animals. In addition, separation of lipid classes by thin layer chromatography indicates that triglyceride resynthesis from labeled oleic acid is proceeding similarly in both treated and control animals. Transfer of resynthesized triglyceride from the intestinal mucosa into the closed sac was also measured and no significant differences between control and treated animals were noted. The lack of an in vitro effect of TCDD exposure on lipid absorption in the mouse intestine suggests that the observed in vivo reduction of lipid absorption is due to a reduced luminal availability of labeled triolein. Possible sites of TCDD interaction that could adversely affect luminal fat processing include reduced pancreatic lipase activity and/or reduced altered bile salt production.

Proposed Course: We plan to concentrate our efforts in fiscal year 1983 on follow-up studies based on our animal models that we are developing for monitoring the regulation of lipid assimilation. Conceivably, at least three or four lines of

investigation follow from our current observations. First is the examination of the metabolism of the lipid components of the lymph lipoproteins, e.g. the chylomicra and VLDL triglycerides, cholesterol and cholesterol ester. Several time course experiments are envisioned in order to follow the removal of these lipids from the serum by the liver and peripheral tissues, such as the heart, adrenals and adipose tissue. Proper selection of lipid precursor allows for the absorption and subsequent resynthesis and labeling of lipid by the intestinal epithelium. This same technique can be employed to analyze the absorption, esterfication, transport and metabolism of lipophilic vitamins such as retinol and also lipophilic toxins. A low concentration acrylamide gel system can be used to examine the transition of a component from one serum lipoprotein to another, e.g. chylomicron to albumin or retinol binding protein. Second, a more detailed examination of lipoprotein lipase will be performed to investigate the sensitivity of the enzyme to heparin after TCDD treatment, the time course of release and also the tissue distribution of this enzyme. In vitro experiments could also employ lymph lipoproteins from control or TCDD-treated animals mixed with serum from control or TCDD-treated animals. A third avenue of investigation is the possible altered role of the liver in the clearance of the chylomicron remants and other lipoproteins after TCDD treatment. Both morphological and biochemical approach would be used to examine changes in the binding capacity of the TCDD-treated liver membranes. In an analogus study, we are interested in changes in the intestinal cell membrane proteins, of special interest are changes in the glycoproteins of the absorptive cell plasma membranes. Lastly, we are interested in pursuing the changes in lipid mobilization and synthesis in the two strains of mouse model. Since the lipids do readily accumulate in the liver and not the serum, it is of concern to examine the regulation of free fatty acid between the adipose tissue and the liver.

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, the regulation of the intestinal epithelial metabolism may be expected to be more complicated than other tissues because of the constant cell turnover with a short half-life and the additional function of absorption of essential nutrients. Our studies with the lipophilic toxin, TCDD, may provide an interesting model for probing the regulation of lipid assimilation.

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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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70200 -09 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms for Regulating the Intracellular Bioavailability of Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Bruce A. Fowler Research Biologist LP NIEHS

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Pharmacology

SECTION

Receptor Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms which regulate the intracellular bioavailability of metals such as lead and cadmium have been studied in both mammals and marine invertebrates. High affinity cytosolic lead-binding proteins of 63,000 and 11,5000 daltons from kidneys of rats have been partially purified by gel and anion exchange chromatography, electrophoresis, saturation and sucrose density gradient analysis. These molecules were found to exhibit dissociation constants (K_d) for lead of 10^{-8} M but addition of cadmium and zinc displaced 40-80% of the bound lead indicating an alteration of binding Pb capacity by these metals. The biological role of metallothionein (MT) in mediating the intracellular bioavailability of cadmium in renal proximal tubule cells following zinc induction of MT or zinc deficiency showed marked decreases in non-MT bound cadmium from injected CdMT following prior zinc induction of renal MT and increases in non-MT bound cadmium under following zinc deficiency. The binding site of a low molecular cadmium binding protein (CdBP) from oysters which is similar in size to metallothionein (MT) but which contains 7.6% cysteine and binds only 1-2 g atoms Cd/mole protein was also characterized. Scatchard analysis of ^{109}Cd binding to purified CdBP showed a single class of site(s) with an apparent dissociation constant (K_d) of 10^{-7} M for Cd. An SH:Cd ratio of 2:1 for CdBP instead of the 4:1 ratio reported for MT was determined by SH group titration. Circular dichroism studies of CdBP incubated in vitro with a 2-fold excess of Cd or Cu disclosed marked reduction in the positive 259 nm Cd-S bond peak but no changes in other portions of the spectrum. These studies suggest that the lower Cd-binding affinity of CdBP relative to MT stems from the presence of only 2 SH groups at the Cd binding site.

PI:	B.A. Fowler	Research Biologist	LP	NIEHS
Other:	P. Mistry	Visiting Fellow	LP	NIEHS
	P. Goering	NRSA Postdoctoral Fellow	LP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy, x-ray microanalysis, saturation analysis and sucrose denisty gradient analysis of cytosolic metal-binding proteins, Sephadex and DEAE-chromatography, electrophoresis, isoelectric focusing and amino acid analysis of metal binding proteins from mammals and marine sheelfish. Tissue analysis for metals by atomic absorption spectroscopy and gamma emission spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Recent studies from this laboratory have demonstrated the presence of 11,500 and 63,000 dalton ^{203}Pb-binding components in kidney cytosol of rats (Oskarsson et al., Biochem. Biophys. Res. Commun. 104: 290, 1982). Saturation analysis of these two components following partial purification by Sepharose 6-B column chromatography disclosed an apparent dissociation constant (K_d) for Pb^{2+} of approximately 3 x 10^{-8} M for each component. The Pb-binding capacity of the whole cytosol was 43 pmol/mg protein. Sucrose density gradient analysis (SDGA) yielded approximate sedimentation coefficients of 2S and 4.6S for the 11,500 and 63,000 components, respectively. Competition studies with other divalent cations on SDGA showed 40-80% displacement by Cd^{2+} and Zn^{2+} and little or no displacement by La^{2+}, Cu^{2+}, and Mg^{2+}. Further detailed competitive binding studies on the partially purified components showed that Pb^{2+} concentrations above 13 μM produced a marked increase in binding of Pb^{2+} by the 11,500 component. This cooperative binding effect was also observed for both components in the presence of Fe^{2+}, In^{3+} and Sn^{4+}. The results of these studies indicated that the previously reported cytosolic Pb-binding components of rat kidney are saturable and possess a high affinity for Pb^{2+}. In addition, the binding capacity of these components appears to be altered by the presence of other metal ions and high concentrations of Pb^{2+}. Such factors may thus influence the binding and bioavailability of Pb^{2+} in vivo.

The mechanism of cadmium-metallothionein (CdMT) nephrotoxicity has been studied in rats using an acute dose regimen. A single dose of CdMT (0.6 mg Cd/kg body weight, i.p.) induced significant increases in urine volume, creatinine excretion and low molecular weight protein (RNAase) excretion within 8 hr of treatment. The low molecular weight proteinuria was not due to increased glomerular filtration for RNAase/creatinine ratios were significantly elevated. Prior induction of renal MT by treatment with zinc (2 mg Zn as $ZnSO_4$, i.p., 16 hr prior to CdMT injection) inhibited the alterations in glomerular filtration and low molecular weight protein reabsorption induced by CdMT. The earliest ultrastructural change observed was an increase in the number of small lysosomes in the proximal tubule cells after 1 hr; this was followed by an increase in the numbers of small apical vacuoules and a further increase in small lysosomes at 4 and 8 hr. By 4 hr, RNA synthesis and lysosomal cathepsin D activity were significantly decreased. In vitro studies showed that the latter was not due to direct inhibition by CdMT or Cd^{+2}. Metabolism studies using ^{3}H- and ^{109}Cd-labeled CdMT indicated that CdMT is rapidly degraded by proximal tubule cell lysosomes with subsequent release of

Cd^{+2} into non-thionein components of the cytoplasm of the cell. These data suggest that CdMT nephrotoxicity occurs as a result of Cd^{+2} toxicity within the cell following degradation of CdMT in proximal tubule cell lysosomes. Low molecular weight proteinuria develops as a result of an inhibition of tubular protein reabsorption and degradation due to an inhibition of normal lysosome formation and the fusion of apical pinocytotic vesicles with primary lysosomes. Prior induction of renal MT by Zn treatment appears to protect against these effects by reducing the non-MT binding of Cd^{+2} within the proximal tubule cells.

In order to understand the role of MT in regulation of Cd in kidney, a study was undertaken to examine, in detail, the quantitative distribution of Cd, Zn, and Cu among cellular compartments of the kidney after chronic exposure of male rats to low levels of cadmium in the drinking water. Because of documented Cd-Zn interactions, the pattern of metal distribution was measured as a function of the zinc status of the animals. Rats received a stock diet and 100 µg Cd/ml of drinking water for 30 days followed by 14 days of a semipurified Zn sufficient (Zn^{+}) or Zn deficient (Zn^{-}) diet and distilled water. General features of metal distribution in the kidney included the finding of comparable concentrations (µg/g of protein) of Cd in cytosol, membrane, and mitochondrial fractions. Although less than 5% of cytosolic Cd is not bound to metallothionein (MT), 3-5 times as much non-MT-Cd is present in other fractions. Zinc deficiency increases non-MT-Cd in the membrane fraction. Quantitation of the Cu and Zn distribution in high molecular weight, superoxide dismutase, and metallothionein regions of the profiles of metals from Sephadex G-75 chromatography shows that, in general, metal content only changes in the MT fraction after Cd treatment. Zinc deficiency only lowers MT-Zn and to some extent MT-Cu. It does not affect the MT-Cd concentration. Kidney normally contains a substantial amount of Zn, Cu-MT. Zinc deficiency greatly reduces its Zn and Cu content without affecting other cellular pools. When rats are exposed to 100 µg Cd/ml drinking water, much new MT synthesis occurs to bind Cd and additional Zn and Cu. After exposure to 20 µg Cd/ml for 30 days, however, basal synthesis of MT is not enhanced. Binding of Cd to MT occurs with the loss of Zn and Cu from the protein. These results suggest new approaches to the subtle questions of chronic Cd toxicity. They will be discussed in relationship to possible normal roles of MT in kidney.

Previous studies (Ridlington and Fowler, Chem.-Biol. Interact. 25: 127, 1979) have shown that the American oyster (Crassostrea virginica) produces a low molecular weight cadmium-binding protein (CdBP) similar in size to metallothionein (MT) but which contains 7.6% cysteine and binds only 1-2 g atoms Cd/mole protein. Scatchard analysis of ^{109}Cd binding to purified CdBP showed a single class of site(s) with an apparent dissociation constant (K_d) of 10^{-7} M for Cd. Addition of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to CdBP followed by separation of protein bound and free ionic Cd on small Sephadex G-25 columns demonstrated displacement of Cd from CdBP. An SH:Cd ratio of 2:1 for CdBP instead of the 4:1 ratio reported for MT was determined by this method. Incubation of CdBP with EDTA (1.3 mM) showed little release of Cd from the protein except when DTNB was added. Circular dichroism studies of CdBP incubated in vitro with a 2-fold excess of Cd or Cu disclosed marked reduction in the positive 259 nm Cd-S bond peak but no changes in other portions of the spectrum. In addition, CdBP isolated from oysters collected in areas with greater human activity and possessing higher tissue burdens of Cu from in vivo exposure showed similar circular dichroic properties. These

studies suggest that the lower Cd-binding affinity of CdBP relative to MT stems from the presence of only 2 SH groups at the Cd binding site, but that like MT, these groups inhibit EDTA chelation of Cd from the protein. Addition of excess Cd or Cu resulted in the formation of an optically inactive complex, at 259 nm which appears to have an SH:Cd or Cu ratio of 1:1.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize the intracellular molecular mechanisms which regulate the intracellular bioavailability of metals such as lead and cadmium by combined ultrastructural and biochemical techniques. At present, high affinity cytosolic metal-binding proteins appear to be of extreme importance in this regard, particularly at low level exposures in that they are capable of binding the vast majority of these metal ions within cells and thereby preventing their interaction with sensitive metabolic processes.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 71000-04 LP

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Estrogen and Androgen Action in Liver

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
G. Lucier Research Chemist LP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Receptor Pharmacology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	3.0	4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

PI:	G. Lucier	Research Chemist	LP	NIEHS
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	T. Sloop	Biologist	LP	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LP	NIEHS
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	D. Campen	Graduate Student	LP	NIEHS
	A. Vickers	NIH Postdoctoral	LP	NIEHS

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 [illegible] gr[illegible]tation and exchange assays.

IV. Elucidate [illegible] role of HCLA sites in the [illegible] studies compa[illegible] rate of uptake and nuclear [illegible] of plexes in re[illegible] o the quality and quantity [illegible] te of HCLA sites [illegible]oduced by partial purificat[illegible] ic the nat[illegible] animal. Techniques are t[illegible]

[illegible] role that estrogen and andr[illegible] ep[illegible] [illegible]emical and physiological resp[illegible] t. [illegible]ding estrogenically active xenob[illegible] [illegible]are serum triglycerides, production of s [illegible]omponents of cytochrome P-450, analysis of protei [illegible]s by two-dimensional gel electrophoresis, and anal[illegible] [illegible] in vitro translation assays.

[illegible]y estrogen and androgen responsive proteins for the purp[illegible] [illegible]s for steroid hormone action in liver

VII. Evaluate the role of hepatic estroge[illegible] tor in the promotion of liver tumors by synthetic estrogens. Diethynitr[illegible] will be used as the initiating agent and ethinyl estradiol as the promot[illegible] t. GGT positive foci and nodules will be used to quantitate neoplasia.

MAJOR FINDINGS AND PROPOSED COURSE: [illegible] sidered target organ f[illegible] stro-gens a[illegible] contains estrogen recepto[illegible] . A[illegible] ond c[illegible] estrogen b[illegible] pro-teins. [illegible]ion exhibit higher capacity, lower affinity [illegible] binding than [illegible]s also [illegible] detected in liver. HCLA sites bind steroid[illegible] ogens and es but not [illegible]steroidal estrogens or p[illegible] steroids. [illegible] s undergo se[illegible]er-ential [illegible] such that adult male level[illegible] e 10-fold hig[illegible] n adult fema[illegible]els. Normal [illegible]e levels of HCLA sites re[illegible] e neonatal e[illegible] o androgens d[illegible] g a brief [illegible]tical period of development. The le[illegible] tes are affecte[illegible] by androgens and estrogens in an age-dep[illegible]ent [illegible] tion of testosterone (T) to castrated males during the brief pe[illegible]) imprints for sex differentiation of HCL[illegible] sites, whereas [illegible] [illegible]ther times does not Treatment with DES [illegible]zearalenol is [illegible] ri[illegible] development of nor[illegible] levels at any age. [illegible]ver, DES given [illegible] produces age-specific [illegible]fects. When tre[illegible] DES on days [illegible] males fail to develop al levels of H[illegible] DES administrat[illegible] and 13 has no effects The level of [illegible] n adult castrate[illegible] eminized by DES and est but not zear[illegible] e studies demons[illegible] [illegible]e-specific hormonal regu[illegible] of hepatic [illegible] ich can be disr[illegible] agents that alter [illegible] e hormo environment [illegible]ages of developme[illegible]

HCLA sites [illegible] of two classes of proteins: moderate a[illegible] (dissocia-tion consta[illegible] 5 μM and 0.24 μM) estrogen binding sites [illegible] to male cytosol and [illegible] ity, non-saturable estrogen binding site[illegible] in both sexes. Neo[illegible] stration causes a reduction in the concentrat[illegible] HCLA sites in the subse[illegible] dult male. Furthermore, the moderate affinity [illegible]es detected by Scatchard a[illegible] ysis in adult male liver are not observed in neonatal castrates.

Cell free nuclear translocation assays demonstrate that nuclear uptake of cytosolic receptor-ligand complexes is more efficient in females than in males. This sex difference in nuclear uptake can be minimized when the concentration of the ligand is increased to a level necessary to saturate the estrogen receptor in the presence of HCLA sites. Nuclear uptake of receptor ligand complexes in neonatally castrated males (deficient in HCLA sites) is similar to that seen in the adult female. Elevations of serum triglyceride following estradiol exposure have been monitored as an indicator of hepatic responses to estrogen. Our studies have shown that the female liver appears more responsive to estrogen exposure than the male liver. While a dose of 20-30 µg estradiol/kg body wt/day was sufficient to produce a three- to fourfold increase in the concentration of triglyceride associated with the very low density lipoprotein fraction in females, 100 µg estradiol/kg body wt/day was needed to obtain a similar response in males. However, following neonatal castration, estrogen responsiveness in the subsequent adult male rat was similar to that in females suggesting a role for neonatal androgens in regulating sex differences in hepatic estrogen action.

The isolated perfused rat liver was investigated as a potential model to analyze binding of ^{3}H-17β-estradiol (E_2) to cytosolic and nuclear estrogen receptors. Viability of the isolated perfused liver was monitored by measuring leakage of cytosolic enzymes into the perfusate. These studies indicated that the liver remained viable for at least a 90-min perfusion period although significant decreases in cytosolic estrogen receptor concentrations occurred during this perfusion period. Estrogen receptor loss was minimized by supplementing the red blood cell free perfusion medium with 5 µg insulin/ml. Uptake of ^{3}H-E_2 by hepatic cytosolic estrogen receptors of the isolated perfused liver was rapid as measured by partial purification of radiolabeled ligand receptor complexes after varying times of perfusion. Peak liver concentrations of receptor bound E_2 were achieved 15 min after the onset of perfusion when using livers from either male or female rats. After 15 min, radiolabeled cytosolic ligand receptor complexes decreased rapidly reaching lowest concentrations at 60 min. Radiolabeled salt extractable nuclear binding sites increased up to 30 min and then decreased slightly between 30 and 90 min. Both 4S and 5S forms of nuclear binding sites were detected in the isolated perfused livers as evaluated by sedimentation analysis on 5-20% sucrose density gradients. Concentrations of radiolabeled cytosolic and nuclear receptors were greater in females than males at all perfusion periods examined when the initial concentration of ^{3}H-E_2 was 4 nm. Sex differences in receptor uptake were not as great when higher concentrations of ^{3}H-E_2 were added to the perfusion medium. These studies suggest that the isolated perfused liver is a suitable model to investigate short term uptake of estrogens by cytosolic and nuclear receptors.

The mycotoxin zearalenone and some of its derivatives possess estrogenic activity. Zearalanol (P-1496) was the most effective derivative in displacing radiolabeled E_2 from the hepatic cytosolic estrogen receptor (Powell-Jones et al., Mol. Pharmacol. 20: 35-42, 1981). The estrogenic properties of P-1496 in liver were examined further by evaluating the in vitro binding of radiolabeled P-1496 to estrogen receptor. Scatchard analysis revealed that the apparent K_d was 0.5 nM and the number of binding sites were 37 ± 13 fmol/mg cytosol protein in female rats. Analysis of the binding on sucrose gradients showed two peaks of radioactivity with sedimentation coefficients of approximately 4S and 8S. The peak corresponding to 8S was completely displaced by 100-fold excess of P-1496 or E_2 and is thought to be cytosolic estrogen receptor. Binding in the 4S region was

partially displaced by 100-fold excess P-1496 but not E_2. Translocation of bound receptor to the nucleus and binding to DNA-cellulose were also examined. Moreover, in vivo studies were conducted. Ovariectomized rats which had been injected daily with P-1496 (2 mg/kg) for two weeks exhibited increased concentrations of serum triglycerides. Separation of the different classes of lipoproteins followed by quantitation of their triglycerides showed that the increase was associated solely with the very low density lipoprotein (VLDL) fraction. Dose-response studies suggested that a daily dose greater than 0.5 mg/kg was required to produce signiicant increases. The time course of the response showed similarities to that obtained for E_2 in that significant changes in VLDL concentrations were not observed until 7 days of treatment. P-1496 also affected the synthesis of several proteins in a manner similar to E_2. These proteins were synthesized in vitro by hepatocytes isolated from treated animals using ^{35}S-labeled methionine and cysteine and subsequently analyzed by two-dimensional gel electrophoresis. Our studies indicate that P-1496 binds to liver estrogen receptor as well as E_2 and that treatment of rats with P-1496 produces selective changes in hepatic protein synthesis.

Previous reports have demonstrated the presence of moderate to high affinity binding for androgens in the cytosol of livers from male rats. This binding was significantly lower in female rats or in immature rats of either sex. The hepatic androgen binding protein, which sedimented at approximately 4S on sucrose density gradients, has been called a receptor which mediates the actions of androgens in the liver. The experiments in the present study were designed to evaluate the hepatic androgen binding protein for characteristics which have been attributed to receptors in other tissues and to correlate the presence of androgen binding protein with androgen induction of hepatic drug metabolism.

In the current studies, we have shown that cytosol from the livers of male rats bound ^3H-dihydrotestosterone (^3H-DHT) and translocated this steroid ligand to the nucleus in a time- and temperature-dependent manner. Cytosol prelabeled with ^3H-DHT, when passed over a column of denatured DNA cellulose, eluted in three radioactive peaks. Two of these peaks were absent when cytosol from livers of female or hypophysectomized males was used. In addition, the presence of high concentrations of hepatic androgen binding correlated well with the ability of androgen to induce ethylmorphine N-demethylase, a marker of microsomal cytochrome P-450-dependent drug metabolism. Values for both parameters were higher in males than in either females or hypophysectomized males. Testosterone treatment induced both parameters in ovariectomized females, and E_2 repressed both in males. However, testosterone treatment failed to induce hepatic androgen binding in hypophysectomized males and immature males, both of which are also unresponsive to androgen induction of drug metabolism. The results suggest that one or more hepatic cytosolic androgen binding proteins possess several characteristics associated with steroid receptors in reproductive tract tissue. Furthermore, this binding may be implicated as a mediator for the androgen induction of at least one component of hepatic drug metabolism.

Our results described above had demonstrated protein(s) which bind androgens with high affinity in liver cytosol of male rats. The androgen binding sites (ABS) of male rat liver translocates androgen to the nucleus, bind to DNA, and their presence correlates with several androgen-responsive functions of rat liver suggesting that these sites may act as androgen receptor. Our results also indicate

that expression of the ABS in liver cytosol of the adult male rat depends on exposure to androgen during a brief critical period in neonatal life. Male rats castrated on day 42 exhibit concentrations of hepatic ABS similar to those of intact males. However, ABS are completely absent in immature rats of either sex, intact females, or males castrated on day 1 of life. Treatment of neonatal castrate males with testosterone propionate (TP) on days 2, 6, 9 and 13 restores the concentrations of ABS to those of adult intact males, although TP given on days 20, 23, 27 and 30 has no effect on sex differentiation of ABS. Normal high concentrations of hepatic ABS can also be restored with continuous adult exposure to testosterone. Neonatal treatment of female rats results in development of ABS only if the females are ovariectomized prior to puberty. E_2 treatment of male rats reduces the concentration of ABS equally in either intact males or neonatal castrate males which received TP. Development of hepatic ABS is also affected by neonatal exposure to environmental estrogenic chemicals. Neonatal treatment of intact male rat pups with diethylstilbestrol (DES) subsequently inhibits the expression of hepatic ABS in adult life. Treatment of these rats as adults with TP partially reverses the DES inhibition. When DES treated male pups are supplemented with neonatal TP, reversal of the DES inhibition is seen. Similar effects are observed if the mycotoxin α-zearalenol (P-1496) is used in an identical treatment regimen. These results suggest that an important sex difference in hepatic function in adult rats is determined by the hormonal environment during a critical period shortly after birth and this hepatic sexual dimorphism can be markedly altered by exposure to environmental agents early in life.

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 studied will include molecular aspects of the estrogen-induced production of triglycerides, VLDL (very low density lipoporteins), protein synthesis as analyzed by two-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.

A study is in progress which is attempting to evaluate the role of hepatic estrogen receptor in the promotion of liver tumors by synthetic estrogens. Tumor promoting activity of estrogens occurs primarily in the periportal region of liver so we are investigating estrogen receptor action in isolated cells from this region including characterization of newly synthesized unusual proteins at various time points following estrogen treatment of initiated cells.

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 important 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 hepatoxins. 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. Of particular importance are the mechanisms whereby estrogenically-active chemicals influence the carcinogenic processes.

PUBLICATIONS

Thompson, C., Hudson, P.M., and Lucier, G.W.: Correlation of hepatic estrogen receptor concentration and estrogen-mediated elevation of very low density lipoproteins. Endocrinol. 112(4): 1389-1398, 1983.

Lui, E.M.K., Slaughter, S.R., Philpot, R.M., and Lucier, G.W.: Endocrine regulation of cadmium-sensitive cytochrome P-450 in rat liver. Mol. Pharmacol. 22: 705-802, 1982.

Sloop, T.C., Clark, J.C., Rumbaugh, R.C., and Lucier, G.W.: Imprinting of hepatic estrogen binding proteins by neonatal androgens. Endocrinol. 112(5): 1639-1646, 1983.

Rumbaugh, R.C., Clark, J.C., McDaniel, O.S., and Lucier, G.W.: Feminization of hepatic estrogen binding proteins by ectopic pituitary. Endocrinol. 112(4): 1361-1369, 1983.

Thompson, C., and Lucier, G.W.: Hepatic estrogen responsiveness: possible mechanisms for sexual dimorphism. Mol. Pharmacol., in press, 1983.

Sloop, T.C., Sherman, B., Fisher, S., and Lucier, G.W.: High affinity estrogen binding in cytosol of winter flounder liver. Mount Desert Island Biological Laboratory Bulletin Vol. 22, in press, 1982.

Lucier, G., Lui, E., and Powell-Jones, W.: Regulation of hepatic enzyme development by steroids. Environmental Factors in Human Growth and Development. Banbury Report 11, 1982, pp. 137-150.

Lamartiniere, C.A., Sloop, T., Clark, J., Tilson, H.A., and Lucier, G.W.: Organizational effects of hormones and hormonally-active xenobiotics on postnatal development. 12th Conference on Environmental Toxicology, USAF A FAMRL-TR-81-149, 1982, pp. 96-121.

Lucier, G.W., and Rumbaugh, R.C.: Endocrine host factors affecting experimental carcinogenesis. Host Factors and Experimental Carcinogenesis. International Agency for Research on Cancer. In press.

Lucier, G.W., Rumbaugh, R.C., Sloop, T.C., and Thompson, C.L.: Sex differentiation of liver protein synthesis. Prevention of Congenital and Mental Defects. Alan Liss Inc. In press.

Lucier, G.W., Thompson, C.L., Sloop, T.C., and Rumbaugh, R.C.: Sex dimorphism of rat hepatic estrogen and androgen action. Sex Differentiation Basic and Clinical Aspects. Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80001-11 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microsomal Mixed-Function Oxidase Systems: Specificity and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard M. Philpot Research Chemist LP NIEHS

COOPERATING UNITS *(if any)* Dept. of Biochemistry, Univ. of Vermont; Dept. of Biochemistry, Scripps Clinic and Research Foundation; Dept. of Anatomy, School of Veterinary Medicine, University of California, Davis, CA

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:
6.5	3.5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal monooxygenase systems from various sources. Present work involves the quantitation of several isozymes of cytochrome P-450 in rabbit liver and lung microsomal preparations and the inductive and repressive effects of various environmental chemicals on the synthesis of these isozymes. Components of the monooxygenase systems are being examined on the basis of catalytic activities as determined in purified and microsomal systems. The enzymes are being quantitated by immunochemical methods. The long range objectives of this research are to: 1) determine the effects of changes in the profiles of isozymes of cytochrome P-450 on overall activity in microsomal preparations and to relate these changes to effects in vivo; 2) to determine differences in the responses of monooxygenase systems from different tissues to exogenous chemicals; and 3) to determine differences in the mechanisms that control the synthesis of isozymes of cytochrome P-450 in rabbit liver and lung.

PHS 6040 (Rev. 1/83)

GPO 895-100

PI:	Richard M. Philpot	Research Chemist	LP	NIEHS
	Barbara A. Domin	Staff Fellow	LP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Different isozymes of cytochrome P-450 are purified from rabbit liver and lung by methdos developed in this laboratory. Quantitation of these enzymes is accomplished by methods that have been derived by modification of existing techniques such as "Western blotting".

MAJOR FINDINGS AND PROPOSED COURSE: 1) Treatment of rabbits with polycyclic aromatic hydrocarbons results in the induction of the synthesis of at least two isozymes of cytochrome P-450 in the liver and repression of the synthesis of at least two others. This finding explains the time difference between changes in total cytochrome P-450 and increases in activity in the liver following treatment with compounds like benzo(a)pyrene and tetrachlorodibenzo-p-dioxin. Increases in the concentrations of isozymes that catalyze the activity being monitored occur with the same time courses as does the increase in activity. The decreases observed in the concentrations of other isozymes also occur with the same time course and this accounts for the lack of change seen in the concentration of total cytochrome P-450 during the first 16-18 hours following treatment. In contrast to the liver, only one isozyme increases in concentration in the lung following treatment of rabbits with polycyclic aromatic hydrocarbons and little or no decrease is detected in the concentrations of the isozymes whose content is diminished in the liver.

2) Isozymes of cytochrome P-450 can be quantitated by a modification of the "Western blotting" technique. This method has proven to be very useful but is not without problems. We have shown that the technique is dependable for some isozymes, particularly form 2, but not for others. The major problems encountered have to do with a lack of reproducibility obtained with some of the purified standards. The low concentrations of standard required for the assay are very sensitive to the concentration of sodium dodecyl sulfate and reducing agent used and to the length of time they are subject to heating. Our findings indicate that optimal conditions will have to be established independently for each isozyme of cytochrome P-450 before the assay can be used with confidence.

In an effort to avoid some of the problems inherent with the "Western blotting" technique, we have developed a direct quantitation assay that eliminates the requirement for electrophoresis. In this assay, microsomes are bound directly to nitrocellulose using a DNA-RNA "hydridot" apparatus. For cytochrome P-450, form 2, we have demonstrated that this assay gives the same results as single radial immunodiffusion and "Western blotting". The advantages of the direct immunochemical assay are that up to 100 samples can be read in 2 hours and that the sensitivity is in the range of 1-2 fmol.

3) Cytochrome P-450, form 6, has been detected in lungs from untreated rabbits. The presence of this isozyme is important with respect to the metabolism of benzo(a)pyrene, a pulmonary carcinogen. Previous to this finding it was thought that benzo(a)pyrene was metabolized in rabbit lung by forms 2 and 5.

Modulation of isozymes of cytochrome P-450 in rabbit liver and lung by exogenous chemicals shows some marked differences. The major isozymes in the lung, forms 2 and 5, are minor forms in the liver. However, the hepatic concentrations of forms 2 and 5 are markedly increased by treatment of rabbits with phenobarbital, a compound that has no effect on the lung. Polycyclic aromatic hydrocarbons increase the hepatic concentrations of forms 4 and 6 and decrease the concentrations of forms 2 and 5. In the lung, only the concentration of form 6 is increased and the concentrations of 2 and 5 are not altered. We now plan to investigate these changes with respect to levels of mRNA to establish whether they all involve induction or repression. Ultimately, efforts will be made to determine the molecular reasons for the differences observed in the control of synthesis of isozymes of cytochrome P-450 in rabbit liver and lung.

PUBLICATIONS

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. In Sato, R. and Kato, R. (Eds.): Microsomes, Drug Oxidations and Drug Toxicity. New York, Wiley-Interscience, 1982, pp. 487-494.

Wolf, C.R., Statham, C.N., McMenamin, M.K., 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. 22: 738-744, 1982.

Slaughter, S.R., Statham, C.N., Philpot, R.M., and Boyd, M.R.: Covalent binding of metabolites of 4-ipomeanol to rabbit pulmonary and hepatic microsomal proteins and to the enzymes of the pulmonary cytochrome P-450-dependent monooxygenase system. J. Pharmacol. Expt. Therapeu. 224: 252-257, 1983.

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 a mammalian enzyme. Chemico-Biol Interactions, in press, 1983.

Robertson, I.C.G., Serabjit-Singh, C.J., Croft, J.E., and Philpot, R.M.: The relationship between increases in the hepatic content of cytochrome P-450, form 5, and in the metabolism of aromatic amines to mutagenic products following treatment of rabbits with phenobarbital. Molec. Pharmacol., in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80002-13 LP

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Enzymes Metabolizing Chemicals: Effectors of These Systems

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)
(Name, title, laboratory, and institute affiliation)

James R. Fouts	Research Pharmacologist	LP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

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. Enzyme systems 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. Enzyme 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). 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.

PI:	James R. Fouts	Research Pharmacologist	LP	NIEHS
	Theodora R. Devereux	Research Biologist	LP	NIEHS
Other:	Richard M. Philpot	Research Chemist	LP	NIEHS
	John R. Bend	Chief	LP	NIEHS
	James Mathews	Staff Fellow	LP	NIEHS
	Kandiah Sivarajah	IPA	LPFT	NIEHS
	Thomas E. Eling	Research Chemist	LPFT	NIEHS

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 various substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques have been developed to disperse and separate lung cell types in order to localize and study drug metabolism in individual cell populations. Past research was directed toward obtaining relatively pure populations of alveolar type II cells (80-90% purity) and Clara cells (60-70% purity) since these cell types contain well-developed endoplasmic reticulum (where mixed-function oxidase activity seems to occur). The alveolar type II cell fraction contains 7-ethoxycoumarin (7-EC) 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 homogenate from whole lung. 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. 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 (PNA) in Clara and type II cells has been examined with some differences noted between the two cell types. There was more NADH-dependent metabolism of 7-EC and PNA in Clara than in type II cells. An antibody to NADPH cytochrome P-450 reductase inhibited 7-EC deethylation and PNA demethylation 90% in microsomes of type II cells and whole lung, but only 50% in microsomes of Clara cells. These data suggest more involvement of cytochrome b_5 in the metabolism of 7-EC and PNA in Clara cells than in type II cells. Also, an antibody to cytochrome

$P\text{-}450_2$ inhibited only 60-70% of 7-EC and PNA dealkylation in Clara cell microsomes, but it inhibited 90% of these metabolisms in the microsomes of type II cells and whole lung indicating the possibility of another, as yet, unidentified cytochrome P-450 isozyme in the Clara cells.

A project has been started to examine metabolism of endogenous substrates such as arachidonic acid (AA) and steroids by the isolated cell fractions. Although no metabolism of estradiol was detected in the pulmonary cell fractions, a small amount of progesterone metabolism was measured in both Clara and type II cell fractions. Both 16α- and 6β-hydroxyprogesterone as well as an unknown metabolite(s) (probably reduction products) were detected by TLC. In collaboration with Drs. Sivarajah and Eling, metabolism of AA has been demonstrated in the isolated pulmonary cell fractions. In contrast to rat lung, very little prostaglandin synthetase or cooxygenation of benzo(a)pyrene-7,8-diol was observed in type II cell or Clara cell fractions isolated from rabbit lung. However, hydroperoxy-eicosatetranoic acid metabolites were detected when microsomes of Clara cells, type II cells or whole lung from rabbits were incubated with AA. Further research is being done on this project to characterize the lipoxygenase pathway in these isolated pulmonary cells.

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. In collaboration with Drs. Mathews and Bend, we plan to measure the binding of 1-aminobenzotriazole to cytochrome P-450 in individual pulmonary cells in order to quantify the cytochrome P-450 in these cells.

A project is being started to isolate pulmonary endothelial cells from rabbit lung. These cells may have some forms of cytochrome P-450, and are certainly important in the transport of chemicals and metabolites through the lung.

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. How the lung (and individual cells within the lung) handles xenobiotics may determine whether or not these chemicals are ultimately harmful. 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. An understanding of factors which modify or alter these processes will contribute to a rational basis for assessment of the risks to health in the environment.

PUBLICATIONS

Jones, K.G., Holland, J.F., and Fouts, J.R.: Benzo(a)pyrene hydroxylase activity in enriched populations of Clara cells and alveolar type II cells from control and β-naphthoflavone pretreated rats. Cancer Research 42: 4658-4663, 1982.

Jones, K.G., Holland, J.F., Foureman, G.L., Bend, J.R., and Fouts, J.R.: Xenobiotic metabolism in Clara cells and alveolar type II cells isolated from rats treated with β-naphthoflavone. J. Pharmacol. Exp. Ther. (In press).

Sivarajah, K., Jones, K.G., Fouts, J.R., Devereux, T., Shirley, J.E., and Eling, T.E.: Metabolism of (±)-benzo(a)pyrene-7,8-dihydrodiol by enriched populations of Clara cells and alveolar type II cells from rat lung: involvement of prostaglandin synthetase and cytochrome P-450-dependent monooxygenases. Cancer Research (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80003-10 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Xenobiotic-Metabolizing Enzyme Activity in Skin

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Dr. James R. Fouts Research Pharmacologist LP NIEHS

COOPERATING UNITS *(if any)*

Biometry Branch, Histology

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Cell Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 polycyclic hydrocarbons and steroids are being investigated. Metabolism of endogeneous substrates by various skin cell fractions is being studied -- e.g. -- steroids, prostaglandins and leukotrienes.

PI:	James R. Fouts	Research Pharmacologist	LP	NIEHS
	Roberta J. Pohl	Research Biologist	LP	NIEHS
	Marguerite Coomes	Staff Fellow	LP	NIEHS
Other:	Rebecca Sparks	Biological Technician	LP	NIEHS
	Fred Talley	Head, Histology	TRTP	NIEHS

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 identified by staining and morphological characteristics at both the light and EM level. Several enzyme activities were measured by classical techniques -- both oxidative and conjugating enzymes were studied.

MAJOR FINDINGS AND PROPOSED COURSE: Epidermal and sebaceous cells freed from skin of hairless mice by enzyme digestions were separated into cell fractions by metrizamide gradients and elutriation. Fractions from elutriation were characterized by morphology and staining and 7-ethoxycoumarin (7-EC) deethylation activity of these fractions was assayed. Early fractions were small cells -- mostly basal -- while later fractions were richer in larger and more differentiated cells. Enzyme activity increased with differentiation. Sebaceous cells had the highest activity for several xenobiotic metabolisms of all cells studied.

Conjugation of the product of 7-EC metabolism umbelliferone (UMB), with both glucuronic acid and sulfate, was studied in mixed cell populations. At low UMB concentrations and at early times, most UMB is conjugated with sulfate. As UMB continues to be formed and over longer periods of time, glucuronic acid conjugation predominates. Most of the UMB formed by intact skin cells in mixed population is not conjugated in the time periods studied (up to 30 minutes). A major limitation to glucuronide formation may be the concentration of UDPGA in intact skin cells, but this does not seem to be the case with sulfate conjugation.

Sebaceous cell metabolism of 7-EC and benzo(a)pyrene is increased by animal treatment with β-naphthoflavone. The specialized sebaceous cell containing glands of the rodent ear canal -- the Zymbal's gland -- were also rich in xenobiotic metabolisms. In both mice and rats, these cells were active in 7-EC metabolism especially after animal treatment with β-naphthoflavone.

The sebaceous cell population from hairless mouse skin could be separated into at least two fractions -- one much richer in lipid than the other -- by differential centrifugation. These two fractions differed markedly in metabolism of 7-EC.

We have studied the metabolism of progesterone and estradiol by a mixed population of skin cells. Neither steroid is metabolized at more than trace levels by this mixed cell population.

This project is being set aside for the present due to lack of manpower -- but in the future, studies will concentrate on further characterization of the more differentiated cells and the sebaceous cell populations, especially after treatment of the animal with various effectors. Metabolisms of other lipid classes will also

be investigated -- especially the leukotrienes and prostaglandins. The role of xenobiotic metabolism in skin as related to the metabolism of endogenous substrates is virtually unknown.

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. Journal of Pharmacology and Experimental Therapeutics, 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. Chem.-Biol. Interactions, in press.

Pohl, R.J., Coomes, M.W., Sparks, R.W., and Fouts, J.R.: 7-Ethoxycoumarin O-deethylation activity in viable basal and differentiated keratinocytes isolated from the skin of the hairless mouse. Drug Metabolism and Disposition, in press.

Coomes, M.W., Sparks, R.W., and Fouts, J.R.: Oxidation of 7-ethoxycoumarin and conjugation of umbelliferone by intact, viable epidermal cells from the hairless mouse. Biochemical Journal, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80005-09 LP

PERIOD COVERED

NIL--PROJECT TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In vitro Metabolism of Xenobiotics by Selected Marine Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

John R. Bend Chief LP NIEHS

COOPERATING UNITS *(if any)*

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:
0	0	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated because the senior investigator no longer plans to maintain a personal research program at the Mount Desert Island Biological Laboratory.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80007-12 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjugation and Oxidation Pathways for Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John R. Bend Chief LP NIEHS

COOPERATING UNITS *(if any)*

Arrhenius Laboratory of Biochemistry, Stockholm University; Laboratory of Molecular Biophysics, NIEHS; Laboratory of Pulmonary Function and Toxicology, NIEHS

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:
5.3	2.8	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Experiments are conducted 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. Particular attention is being given to the respiratory tract because this is a common site for pollutant-mediated damage. Current research objectives are: 1) to characterize, both stereochemically and kinetically, the biotransformation of several K-region polycyclic arene oxides by purified glutathione transferases of mammalian and marine vertebrate origin; 2) to develop cell selective and isozyme specific suicide inhibitions of rabbit pulmonary cytochrome P-450 for use in studying relationships between chemical metabolism and target cell toxicity in lung; 3) to ascertain the chemical mechanisms by which alkylbenzenes selectively destroy rabbit pulmonary cytochrome P-450; 4) to determine the role of the vasculature in cytochrome P-450-mediated metabolism; 5) to biochemically characterize marine and mammalian NADPH-cytochrome P-450 reductases that exhibit differences in thermal lability and/or redox state; and 6) to determine relationships between total (polar and nonpolar) benzo(a)pyrene metabolite profiles and BP-metabolite-DNA adduct profiles in perfused lungs and cells isolated from the lungs or trachea (both freshly isolated and cultured cells).

PI:	J.R. Bend	Chief	LP	NIEHS
Other:	J. Mathews	Staff Fellow	LP	NIEHS
	E. Cheung	Visiting Fellow	LP	NIEHS
	L. Dostal	NIH-Postodoctoral Fellow	NRSA	NIEHS
	J. Horton	Visiting Fellow	LP	NIEHS
	D. Brier-Russell	Chemist	LP	NIEHS
	G. Foureman	Biologist	LP	NIEHS
	C. Harris	Biologist	LP	NIEHS
	C. Serabjit-Singh	Chemist	LP	NIEHS
	O. Hernandez	Chemist	LMB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Purified enzymes, subcellular fractions of homogenate from various tissues, isolated perfused livers 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-NRM, NMR, mass spectroscopy, etc.) and synthesis of metabolites, when required, are routinely accomplished in collaboration with the Laboratory of Molecular Biophysics (LMB). 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. Selective loss of cytochrome P-450 isozymes in lung is monitored by immunochemical methods and by monitoring enzyme activities associated with specific forms of P-450.

Immunochemical quantitation by Western blotting, presently the most sensitive method for detection of low concentrations of cytochrome P-450 monooxygenase components, can be used to monitor three isozymes of cytochrome P-450 and NADPH reductase in subcellular fractions of rabbit aorta or heart. Immunocytochemical staining of tissue sections can be used to confirm the cellular localization of these isozymes.

Hepatic reductases from rabbit, rat, stingray and little skate are purified by column chromatography. Pure enzymes are compared: immunochemically by Western blotting, enzymatically at various temperatures by cytochrome c reduction and in purified monooxygenase systems, structurally by gel electrophoresis, peptide mapping and fluorometric, UV/vis and E.S.R. spectral analysis of flavin composition and redox states.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Three of the purified glutathione transferases from little skate (Raja erinacea), E-2, E-3, and E-4, had very high V_{max} values (between 10 and 100 μmol/min/mg protein) for pyrene 4,5-oxide (PyO), benz(a)anthracene 5,6-oxide (BO), benzo(a)pyrene 4,5-oxide (BPO) and phenanthrene 9,10-oxide (PO). With both BPO and BO, it was determined that E-2, E-3, and E-4 were stereospecific for a R-configured oxirane carbon. With PO and PyO, product formation was not stereospecific but was highly stereoselective (>95%). Although the absolute configuration of the major products formed from PO and PyO is not

known, behavior on HPLC indicates they are S,S-glutathione adducts. We have also shown that E-4 catalyzes the reaction of GSH with the carcinogenic trans-7,8-dihydrodiol-9,10-epoxide of benzo(a)pyrene.

The stereoselectivity of various rat liver and human glutathione transferases will be investigated with selected polycyclic arene oxides. Also, using an HPLC system that completely resolves the four diastereomeric glutathione adducts formed from styrene 7,8-oxide, the stereoselectivity of the cytochrome P-450-dependent oxidation of styrene to styrene 7,8-oxide will be studied in microsomal and reconstituted monooxygenase systems containing cytochrome P-450.

2) Administration of the suicide inhibitor 1-aminobenzotriazole (ABT) to male New Zealand White rabbits at a dose of 0.1 mmol/kg via the marginal ear vein results in a 42-46% loss of pulmonary cytochrome P-450 and an equal or lower percentage loss of the hepatic enzyme. This spectroscopically measured loss was accompanied by losses in benzphetamine demethylase activity. In incubations of ABT with pulmonary microsomes, up to 70% of the P-450 was destroyed in an NADPH-dependent reaction. ABT does not destroy NADPH-dependent microsomal flavin-containing monooxygenase activity.

Future work will determine the lung cell and cytochrome P-450 isozyme selectivity of the ABT-mediated loss of P-450. Dose-response relationships will be measured to determine if all of the pulmonary P-450 isozymes can be inactivated by ABT or some of its congeners. Finally, attempts will be made to selectively destroy pulmonary P-450 isozymes (versus hepatic P-450) so that the role of circulating carcinogen metabolites in cell-specific DNA alkylation can be studied in rabbit lung in detail.

3) Previous work in this laboratory has shown that each of a series of alkylbenzenes selectively destroys pulmonary cytochrome P-450 (versus the liver). In the case of p-dimethylbenzene (p-xylene) there is evidence that P-450-dependent oxidation of one of its metabolites, p-tolualdehyde, to a reactive intermediate is responsible for at least some of the enzyme loss observed. The homolytic rupture of the aldehydic carbon-hydrogen bond to yield a reactive radical intermediate is a well-documented response to treatment with chemical oxidants. The detection, by ESR spectroscopy, of free radicals produced by the action of pulmonary cytochrome P-450 on the tolualdehyde was therefore attempted. However, no radicals originating from the aldehyde were found. Future studies will attempt to delineate the chemical nature of the reactive metabolite(s) that destroy pulmonary cytochrome P-450.

4) The walls of the vasculature of rabbit liver, heart, aorta and lung exhibit immunofluorescent staining due to the binding of antibodies to cytochrome P-450 isozyme forms 2, 5 or NADPH cytochrome P-450 reductase. Western blots of microsomal fractions from heart and aorta confirm the presence of these enzymes as well as form 6 and its induction by TCDD. We have not detected form 4 in these fractions in disagreement with the reported immunocytofluorescense in the endothelium of lung and kidney.

Future work will include the development of sensitive enzymatic assays that can be used with or without inhibitory antibodies to determine the catalytic

activities of individual isozymes in vascular tisses. Also, we intend to develop and adapt immunocytochemical methods to discern the cellular localization of these enzymes in the intima. We will extend enzymatic and immunochemical analysis to freshly isolated or cultured vascular cells and determine the effects of exogenous cytochrome P-450 monooxygenase modulators on these systems.

5) The thermal lability of the skate cytochrome P-450 reductase relative to that of stingray or mammalian reductase appears to be unrelated to the unusual redox state of the purified enzyme which is isolated as a "semiquinone" (identified by UV/vis spectra). This is suggested by the observation that the stingray enzyme, which is as thermally stable as the mammalian enzyme, is also isolated as a "semiquinone." The ESR spectrum of the skate enzyme does not resemble that of the mammalian semiquinone form.

Future work will determine whether the relative thermal stabilities of these reductases correlate with similarities in structure shown by maps of peptides formed by partial proteolysis. The ESR and UV/vis properties of these enzymes will be further characterized to confirm and extend preliminary observations. The immunochemical similarities of these enzymes will be determined by Western blots of the enzymes and the proteolytically produced peptides. Whether or not there is any correlation between the degree of support of monooxygenase activities by the marine reductases and their thermal lability or unusual redox state will also be determined.

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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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80031-07 LP

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Altered Membrane Function in Xenobiotic Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
John B. Pritchard Research Physiologist LP NIEHS

COOPERATING UNITS *(if any)*
Dr. A. Kleinzeller, Dept. of Physiology, Univ. of Pennsylvania, School of Medicine

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:
3.5	1.7	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 epithelial where the coupled function of apical and basolateral membranes provide 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.

PI: John B. Pritchard Research Physiologist LP NIEHS
Other: Soon Ho Lee Expert Research Biochemist LP NIEHS

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. A new modification using Mg^{++} instead of Ca^{++} has provided BBM membranes with even lower contamination with other membranes. 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. Microsomal vesicle preparations (predominantly plasma membranes) are also used 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 continued to focus on two reabsorptive transport systems localized on the lumenal membrane (BBM) of the renal tubule, glucose (Na^+ dependent), and basic amino acid (proton dependent). The proton driven basic amino acid system is unique. Other amino acids are reabsorbed in the fish via Na-dependent systems analogous to the glucose system, just as they are in the mammal. We have now characterized its specificity and driving forces. It is also sensitive to the xenobiotic pentachlorophenol, a protonophore widely distributed in the environment. This is an important result since it demonstrates that uncouplers may disrupt plasma membrane function in addition to its previously documented mitochondrial effects. Work on the glucose system has focused upon purification and reconstitution

of the carrier protein. In addition to standard biochemical approaches, we have begun an attempt to utilize immunological techniques to accellerate this aspect of our work.

The second major system studied was sulfate transport. We have now shown that the teleost sulfate transport is driven by a pH gradient dependent mechanism at the BLM and that luminal sulfate exit is mediated by anion exchange (for HCO_3 or Cl but not OH). In the mammal where reabsorption rather than secretion predominates, luminal uptake is mediated by Na/sulfate cotransport (this is totally absent in the teleost) and BLM sulfate exit is mediated by exchange for bicarbonate or hydroxyl. BLM systems in teleost and mammal share all properties thus far examined - specificity, ion coupling, kinetics. Each of these systems is distinct from phosphate transport. Current emphasis concerns the 1) driving forces responsible for vectorial sulfate transport in the vesicles and the intact epithelium, 2) control of the membrane events characterized above so that they lead either to net secretion or reabosrption depending upon the state of the animal, and 3) the relative sensitivity of the distinct membrane events to xenobiotics.

We have begun to apply a similar approach to the organic anion transport system - the major system mediating excretion of xenobiotics and their metabolites. It too may be driven by bicarbonate or hydroxyl gradients (i>o) across BLM vesicles. It is as yet unclear whether this system is distinct from the BLM sulfate carrier. At the BBM, different mechanisms appear to be involved.

2. Gill transport: The primary question here was the involvement of anion stimulated ATPase (A -ATPase) in chloride or bicarbonate transport. We have used our highly purified plasma membrane preparation from the gills of low salinity adapted blue crabs as our source of membranes with high A^--ATPase activity. These membranes vesiculate and show anion exchange (HCO_3^-/Cl^-). This exchange process has now been characterized (specificity, inhibition, etc.). It may also be stimulated by ATP. We are now measuring both transport and A^--ATPase activity under a variety of conditions. Thus far, those conditions which stimulate A^--ATPase also stimulate transport.

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.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80032-07 LP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Excretion and Toxicity of Xenobiotics to Marine and Terrestrial Species

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John B. Pritchard Research Physiologist LP NIEHS

COOPERATING UNITS *(if any)*

Dr. M.O. James, College of Pharmacy, Dept. of Medicinal Chemistry, Univ. Florida and Dr. A.L. Krall, Dept. of Biochemistry, Medical Univ. of South Carolina.

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:
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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

PI: John B. Pritchard Research Physiologist LP NIEHS

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.

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. Both control and induced fish produce benzo-ring metabolites of benzo(a)pyrene (BP), metabolites closely related to the carcinogenic mammalian metabolites. We have previously 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 3) that anions (e.g. 2,4-dichlorophenoxyacetic acid ([2,4-D]) effectively retard excretion of BP metabolites, and 4) that the rate limiting step in excretion was production of polar conjugates (sulfate and glucuronide) for subsequent renal transport and elimination.

Based on analysis of the time of appearance and the forms present in urine, it appeared that those xenobiotics converted to sulfate conjugates were eliminated significantly more effectively than those yielding glucuronide conjugates. Both conjugates were more readily eliminated than unconjugated BP or its hydroxylated metabolites. This conclusion was tested directly by following the transport of the fluorescent xenobiotic, 4-methylumbelliferone (4-MU) and its conjugates by isolated flounder tubules in vitro. 4-MU-sulfate was transported twice as well as 4-MU-glucuronide. Both were transported far better than 4-MU itself. Transport was energy dependent and blocked by probenecid. Thus, once conjugated, 4-MU, like BP, is effectively eleminated via active transport on the organic anion system. Furthermore, the sulfate conjugate was a more effective substrate for this system than the glucuronide.

Isolated tubule studies also demonstrated that the kidney was capable of producing the glucuronide, but not the sulfate conjugate from BP-7,8-diol. Another site, presumably the liver must be the source of the sulfate conjugate. This

possibility is currently under investigation. Similar studies with the less well excreted BP metabolites, e.g. the phenols, should provide a more complete picture of the role of anatomic segregation of function in the differential metabolism and excretion of chemically similar BP metabolites. We will then examine the influence of in vivo alterations in conjugation reactions on the excretion of BP metabolites.

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 mitochondrial ATPase activities. We have now demonstrated characteristic decreases in mitochondrial membrane Ca^{++} and proton transport following in vitro DDT and DDA exposure. We are at present attempting to correlate the role of these specific changes with the altered respiration reported previously.

The second collaborative project, with Dr. James, deals with the ability of spiny lobster to metabolize BP in vivo. The lobster has significant quantities of cytochrome P-450, but little in vitro metabolic capacity - presumably because the P-450 reductase is labile and loses activity during isolation. Upon injection into the intact animal, BP was extensively metabolized in vivo. HPLC analysis shows production of phenol, dihydrodiol, and quinone metabolites plus polar conjugates of several of these. Metabolism was decreased and retention prolonged in these cold-blooded animals during the winter. Final analysis of both distribution and metabolism data is now under way.

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.

PUBLICATIONS

Little, P.J., James, M.O., Pritchard, J.B. and Bend, J.R.: Benzo(a)pyrene metabolism in hepatic microsomes from untreated and 3-methylcholanthrene-treated southern flounder, Paralichthys lethostigma. J. Environ. Pathology, Toxicology and Oncology, in press.

Pritchard, J.B. and Renfro, J.L.: Interactions of Xenobiotics with Renal Function. In Weber, L.J. (Ed.): Aquatic Toxicology, Vol. 2, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80037-04 LP

PERIOD COVERED
NIL--PROJECT TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Drug and Xenobiotic Metabolism in the Lungs: Mechanisms and Modifying Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

James R. Fouts	SES	LP	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

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
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Discontinued -- Combined with Z01 ES 80003-10 LP

PHS 6040 (Rev. 1/83)

GPO 895-100

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 within the scope of one laboratory, to cover all aspects of pulmonary function and disease LPFT's work is focused on a few research areas which seem important and are in need of development. Accordingly, LPFT is divided into several 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.

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.

I. 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. One series of studies is concerned with the role of ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis, in the regulation of cell growth. It was found that phorbol esters which are known to have a high binding affinity for the TPA receptor (TPA = 12-O-tetradecanoylphorbol-13-acetate) markedly stimulate ODC activity in tracheal epithelial cell cultures. This also occurred following stimulation with epidermal growth factor (EGF) and high serum concentrations. Parallel investigations showed that TPA and its active analogues which are hyperplasiogenic agents and tumor promoters in mouse epidermis, stimulate colony forming efficiency in tracheal cell cultures, resulting in enhancement of growth. The effects of the phorbol esters could be markedly inhibited by vitamin A analogues (retinoids) which are important factors controling differentiation of a variety of epithelial cell types.

   Studies concerned with cellular differentiation in culture of tracheal epithelial cells from rats, hamsters, and rabbits showed marked species differences in hormone-nutrient requirements as well as substratum requirements. Tracheal cells can differentiate along two principal pathways: the mucous-ciliated cell and the keratinocyte pathway. High serum concentration in the medium stimulates keratinocyte to differentiation which can be prevented by addition of retinoids to the culture medium.

   Studies are also in progress to identify and characterize the various secretory products of tracheal epithelial cells in culture; in particular, extracellular matrix glycoproteins and mucin glycoproteins. Future studies will examine the effect of hormones, retinoids, and extracellular matrix on the biosynthesis of such secretory products, some of which are hoped to be useful markers for measuring cell differentiation of airway epithelial cells.

Studies carried out in collaboration with Dr. C. G. Plopper from the University of California at Davis are concerned with the ultrastructural and histochemical characterization of Clara cells in different size airways of various species. These cells are important as secretory cells and are also important in the metabolism of inhaled or ingested xenobiotics.

II. The Environmental Carcinogenesis Group studies the cellular evolution of neoplasia in airway epithelium using in vivo and in vitro methods. Questions being addressed are: What steps can be discerned during development of epithelial neoplasia? What forces drive the progression? What factors govern the rate of progression? To examine these questions a quantitative tracheal epithelial cell transformation system was developed. It was shown that a variety of carcinogenic agents such as N-methyl-N'-nitro-N-nitrosoquanidine (MNNG), nickel salts and γ-radiation are effective transforming agents. It was also shown that a series of different transformants develop as a result of carcinogen exposure, the neoplastic variant usually occurring late (after 15-25 passages). The quantitative relationships between the late preneoplastic, anchorage independent transformants and the neoplastic transformants are currently under investigation.

An in vivo-in vitro system was used to analyze the evolution of different preneoplastic variants occuring in airway epithelium in vivo following different carcinogenic insults or exposures to tumor promoters. These studies showed that advanced preneoplastic variants (anchorage independent phenotype) develop at very rapid rates following high carcinogen doses but at slow rates following low doses of carcinogen. They also showed that promoting agents did not increase the production of various transformants, rather they seemed to prevent the reversion of transformants. These studies provide new insights into the cellular mechanism of tumor promotion.

One of the unresolved questions of carcinogenesis concerns the possible relationship between mutagenesis and carcinogenesis. In this regard, environmental carcinogens such as asbestos and other durable fibers offer a particularly attractive opportunity to examine the relationship between mutagenesis and carcinogenesis since they are not known to be mutagenic. Studies with glass fibers and chrysotile asbestos were carried out in the Syrian Hamster Embryo Cell system in which mutagenic, chromosomal as well as transforming effects can be studied. These investigations showed that asbestos as well as glass fibers induce morphologic transformation but do not cause point mutations. They also showed that such fibers cause a significant degree of aneuploidy. As in the intrapleural tumor induction studies of M. Stanton, long fibers had considerably more activity (in terms of induction of aneuploidy as well as induction of transformation) than short fibers. These and other experiments suggest that durable fibers interfere with cell mitosis, and their transforming activity may be related to the induction of aneuploidy. Studies with a different type of carcinogen, namely, diethylstilbestrol (DES) showed similar relationships: lack of mutagenic activity, induction of aneuploidy and induction of morphologic transformation. In the case of DES it is presumed that binding of DES to the spindle apparatus interferes with its proper function leading to aneuploidy and transformation. In future experiments the relationship between aneuploidy and transformation will be investigated.

III. The Pulmonary Pathology Group also is involved in studies of asbestos toxicity. A number of studies are underway which to elucidate the mechanisms of asbestos and man-made fiber toxicity, since such fibers not only cause cancer (see above) but also chronic restrictive lung disease (i.e., pulmonary fibrosis. Inhalation studies with chrysotile asbestos in rats showed that within 48 hours after a one hour exposure, lesions can be recognized at alveolar duct bifurcations of exposed rats. These early changes were qualitatively and quantitatively analyzed by electron microscopy and are believed to be the earliest manifestations of the developing interstitial fibrosis.

Other studies were aimed at classifying the mechanism of fiber cytotoxicity. Using the red blood cell as a model to study the membrane effects of asbestos fibers, major differences were demonstrated between chrysotile and crocidolite membrane effects. The data support the hypothesis that chrysotile but not crocidolite causes membrane damage by binding to terminal sialic acid residues on the cell membrane. Similar studies are presently being extended to pulmonary macrophages, one of the important target cell types in vivo.

Pulmonary macrophages are believed to play a key role in the pathogeneis of asbestosis. One of the important questions is: what is the signal which triggers the movement of alveolar macrophages from the interstitium onto the alveolar (duct) surfaces Experiments showed that a chemotactic factor, possibly complement C5a is generated when serum or pulmonary lavage fluid is exposed in vitro to asbestos fibers. Lavage fluid collected from asbestos exposed animals is shown to have increased chemotactic activity for pulmonary macrophages. These and other experiments suggest that asbestos generates a chemotactic signal in the alveolar lining fluid which may be responsible for macrophage migration.

IV. The Prostaglandin Group investigates the production of certain arachidonic acid (AA) derivatives [prostaglandins (PG), hydroxy-fatty acids (HFA), and Leukotrienes (LT)] and studies their role in a number of important physiological and pathophysiological events such as platelet aggregation, inflammation and secretion. Pulmonary tissues produce high amounts of PGs, LTs and HFAs in response to a variety of stimuli. In recent years a number of antithrombotic agents have been developed. One of the most potent one is the compound Bayer 6575. This compound appears to stimulate the synthesis of PGI_2, which is an inhibitor of platelet aggregation. The mechanism of this increased PGI_2 synthesis was investigated and was found not to be due to increased AA release, rather it was found to be related to increased formation of PGH_2, it precursor, and to protecting PGI_2 synthetase from inactivation by peroxides.

Other studies are concerned with the biosynthesis of PGs and LTs in the airways of various species. Marked species differences were found to exist and different cell types (e.g., Clara cells versus type II alveolar cells) showed marked qualitative and quantitative differences in PG synthesis. Dog tracheas were found to produce PGs as well as LTs. Both might be involved in the control of chloride and mucus secretion.

Also being examined is the possible role of LTs in the pathogenesis of asbestos induced pulmonary fibrosis. In particular, the effect of asbestos on the secretions of pulmonary macrophages is being studied. It was found that upon contact with asbestos fibers, pulmonary macrophages release LTB_4 and other as yet unidentified LTs. The release of these mediators might play an important role in the early events of asbestos caused tissue injury.

Many xenobiotics only become cytotoxic, mutagenic, or carcinogenic after metabolic activation by drug metabolizing enzymes such as mixed function oxidases (MFO). Recently it was discovered that prostaglandin synthetase (PGS) can oxidize a number of xenobiotics to reactive (cytotoxic) forms. One of the substrates used is the benzo(a)pyrene intermediate BP-7,8-diol. Comparing the MFO and PGS dependent oxidation of different pulmonary cell populations derived from rats it was found that Clara cells show greater MFO dependent oxidation while type II alveolar cells show greater PGS dependent oxidation. Both hamster as well as human tracheobronchial epithelium were shown to cause PGS dependent oxidation of BP-7,8-diol to anti- and syn-diol epoxides. Related studies are concerned with PGS dependent oxidation of acetaminophen, benzidine and the aromatic amine, 2-aminofluorene, all of which are toxic and/or carcinogenic for the kidney. Efforts are underway to determine the toxic or carcinogenic metabolites formed by PGS.

V. The Biochemical Pathology Group studies the composition function and biosynthesis of pulmonary surfactant. Pulmonary surfactant is composed of phospholipids and proteins lining the alveolar surface. Its major function is to lower the surface tension and to prevent collapse of the alveoli and small airways at low lung volumes. To study the dynamics of surfactant synthesis, secretion and removal in health and disease it is important to be able to compartmentalize the lung surfactant into an intracellular pool (IP) (alveolar type II cells produce and secrete the surfactant) and an extracellular pool (EP). Methods were developed to isolate and quantitate the intra- and extracellular pools. It was shown that the ratio of IP/EP changes as a function of age and as a result of injury induced by environmental agents such as silica. Future studies are designed to elucidate the mechanism of the silica effect resulting in increased IP/EP ratios.

Other studies are concerned with the mechanism through which pulmonary surfactant achieves its alveoli-stabilizing effects. The composition of pulmonary surfactant is complex and although major alveoli-stabilizing properties have been ascribed to dipalmitoylphosphatidylcholine, the most abundant phospholipid of surfactant, the function of the many minor components have not been identified. Using electron spin resonance techniques, the influence of several minor components of surfactant on the fluidity of dipalmitoylphosphatidylcholine have been studied. Phosphatidylglycerol and unsaturated-phosphatidylcholines were demonstrated to have major fluidizing effects possibly accounting for some of the stabilizing properties of pulmonary surfactant at physiological temperatures.

Surfactant is stored in the type II alveolar cells as so called lamellated bodies. Previous studies suggested that these lamellated bodies might be

modified lysosomes. Recent studies with lung slices incubated *in vitro* further support this notion since the secretion of the surfactant from type II cells was found to be accompanied by secretion of lysosomal enzymes. The quantitative relationship between phospholipid and lysosomal hydrolase secretions support the hypothesis that lamellated bodies might be modified lysosomes and that the hydrolases present in them are not associated with surfactant catabolism but rather with events occurring during or after its secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25001-06 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Mutagenesis in Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. C. Barrett Research Chemist LPFT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Environmental Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N.C. 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 and asbestos); and (4) to determine the mechanism by which tumor promoters enhance or induce cell transformation.

Principal Investigator and All Other Personnel Engaged on the Project:

J. Barrett	Research Chemist	LPFT	NIEHS
T. Hesterberg	Postdoctoral Fellow	LPFT	NIEHS
M. Koi	Visiting Fellow	LPFT	NIEHS
M. Oshimura	Expert	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Syrian hamster embryo fibroblasts are exposed to various carcinogeic and mutagenic perturbations and induction of morphological and neoplastic transformation, gene mutation, chromosome aberrations and aneuploidy are all measured in the same target cells as described by Barrett and Ts'o (Proc. Natl, Acad. Sci., USA, 75, 3297- 3301, 1978).

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.

To determine if DES induces cell transformation by a genetic mechanism at the chromosomal level, the effect of DES on structural aberrations and numerical chromosome changes was examined in asynchronous and synchronized cells. Over the concentration range which is optimal for cell transformation, DES failed to induce any increase in chromosome aberrations in the cells. In contrast, significant numerical chromosome changes were observed in DES-treated cultures. The percentage of metaphases with a near diploid chromosome number increased to 19% at 48 hrs, after treatment. By comparison, cells from control cultures contained only 1-2% aneuploid metaphases with a near diploid chromosome number. No significant increase in the number of metaphases with a near tetraploid number (>70) of chromosomes was observed in the DES-treated cultures. DES induced both chromosome loss and gain and no significant difference was detected between the number of hyperdiploid and hypodiploid cells. Chromosome loss or gain was observed for chromosomes in each karyotype group. These findings suggest that DES induces chromosome nondisjunction. Synchronized cell cultures were obtained by first growing the cells in 1% serum and then in 10% serum with hydroxyurea which blocked the cells at the G1/S border. Upon release of the hydroxyurea block, the cells entered into S phase in a very synchronous manner. The cells were treated for 3 hours during one of four time periods after

hydroxyurea release. During the first period the cells were primarily in early S phase, while the second period included cells in late S phase. During the third period most of the cells were in G1 phase, although some mitotic cells were observed. Treatment of the synchronized cells with DES during early or late S phase resulted in little morphological transformation. However, treatment during the third period, when the majority of the cells were in mitosis, resulted in a peak of transformation which was 15 times the level observed in cultures treated in early or late S phase. Treatment during the fourth time period, resulted in a reduced level of cell transformation. Treatment of synchronized culture with DES resulted also in a cell cycle dependent induction of aneuploid cells which paralleled the induction of cell transformation with the greatest level observed following treatment during mitosis. No increase in the percentage of polyploid metaphases or chromosome aberrations were observed in the DES-treated synchronized cells. Parallel dose response curves for cell transformation and aneuploidy induction by DES was observed when the synchronized cultures were treated during the mitotic phase of the cell cycle.

The mechanism by which asbestos, an important environmental human carcinogen, induces tumors is unknown. Stanton and others have shown that mesothelioma induction in rats is related to fiber dimension rather than chemical composition. However, the cellular mechanism of this effect is unknown. We have examined whether asbestos and fiberglass could directly induce morphological transformation of SHE cells in culture and whether the dimensions of the fibers are important in cell transformation, A dose-dependent increase in morphological transformation was observed when cells were exposed to 0.25 to 2 $\mu g/cm^2$ of chrysotile asbestos: Code 100 fiberglass (mean diameter, 0.2 $\mu \pm .01 \mu$) and code 110 fiberglass (mean diameter, 0.8 $\pm$ 0.1 μ) were obtained from Johns-Manville. To further shorten the code 100 fibers, a sample was ground with a mortar and pestel, which did not alter the diameter of the fibers. The mean fiber lengths were as follows: (1) Code 100 before grinding: 16$\mu \pm 2\mu$; (2) Code 100 after grinding: 1$\mu \pm 0.1\mu$ and (3) Code 110: 68$\mu \pm 8\mu$. When transformation frequency was plotted against dose for each fiber, straight lines were formed having slopes close to one, thus indicating a one-hit mechanism for the induction of this change. The unground code 100 fiberglass was about 10 times more potent than the code 110 fiberglass in the induction of morphological transformation. The ground code 100 fiberglass induced no transformed colonies at any of the doses tested. The toxicity of the fibers as measured by a decrease in colony forming efficiency correlated well with the frequency of transformation induced. Our findings correlate with in vivo studies with rats. The tumorigenic potency of mineral fibers appears to be determined by the direct effects of fiber dimension at the cellular level. Doses of asbestos which induced neoplastic transformation did not cause gene mutations but resulted in dose-dependent numerical chromosomal changes. The uptake and accumulation of asbestos fibers in the perinuclear region of the cell may play a role in the induction of heteroploidy. These findings are consistent with aneuploidy induction as a possible mechanism for cancer induction by asbestos.

These studies will be continued to determine the mechanism(s) by which chemicals induce aneuploidy and whether specific cytogenetic and/or molecular changes can

be associated with numerical chromosome changes. The role of other mutagenic events in the multistep process of carcinogenesis will also be studied with this system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fifty to ninety percent of all human cancers are enviromentally 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 mechanism 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 been 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

Boyd, J.A., Barrett, J.C., and Eling, T.E.: Prostaglandin endoperoxide synthetase-dependent co-oxidation of (±)trans-7,8-dihydroxy-7,8-dihydrobenzo(a)-pyrene in C3H/10T 1/2 C18 cells, Cancer Research 42: 2628-2632, 1982.

McLachlan, J,A., Wong, A., and Barrett, J.C.: Morphological and neoplastic transformation of Syrian hamster embryo fibroblasts by diethylstilbestrol and its analogs. Cancer Research 42: 3040-3045, 1982.

Barrett, J.C., Brown, M.T., and Sisskin, E.E.: Deacylation of 12-O-[^{3}H]-tetradecanoyl-phorbol-13-acetate and [3H] phorbol-12,13-didecanoate in hamster skin and hamster cells in culture. Cancer Research 42: 3098-3101, 1982.

Elmore, E., and Barrett, J,C.: Measurement of spontaneous mutation rate at the Na^{+}/K^{+} ATPase locus (ouabain resistance) of human fibroblasts using improved growth condition. Mutation Research 97: 393-404, 1982.

Barrett, J.C., McLachlan, J.A., and Elmore, E.: Inability of diethylstilbestrol to induce 6-thioguanine resistant mutants and to inhibit metabolic coopeation of V-79 chinese hamster cells. Mutation Research 107: 427-432, 1983.

Elmore, E., Kakunage, T., and Barrett, J.C.: Comparison of the spontaneous mutation rates and induced mutation frequencies of normal and chemically transformed human skin fibroblasts. Cancer Research 43: 1650-1655, 1983,

Degen, G.H., Wong, A., Eling, T.E., Barrett, J.C. and McLachlan, J,A.: Peroxidative metabolism of diethylstilbestrol in Syrian hamster embryo fibroblast cell cultures. Cancer Research 43: 992-996, 1983.

Tsutsui, T., Maizumi, H., McLachlan, J.A. and Barrett, J.C.: Possible role of aneuploidy induction in diethylstilbestrol carcinogenicity. Cancer Research, In press, 1983,

Barrett, J.C., Thomassen, D.G., and Hesterberg, T.W.: Role of gene and chromosomal mutations in cell transformation, Ann. N.Y. Acad. Sci. In press,

Crawford, B.D., Barrett, J.C., Ts'o, P.O.P.: Neoplastic conversion of preneoplastic cells in culture: Rate estimate by fluctuation analysis. Mole. and Cell Biol, In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25002-06 LPFT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Carcinogenesis and Promotion Studies with Respiratory Tract Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

P. Nettesheim Chief LPFT NIEHS

COOPERATING UNITS *(if any)*

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:
4.75	2.75	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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; (2) to determine the role of mutagenesis in progression, i.e., in the transition of one transformation stage to the next; (3) to determine the role of tumor promoters in progression, wthether they act mainly through inductive or through selective mechansims; 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.

Principal Investigator and All Other Personnel Engaged on the Project:

P. Nettesheim	Chief, LPFT	LPFT	NIEHS
J. C. Barrett	Research Chemist	LPFT	NIEHS
D. Thomassen	Postdoctoral Fellow	LPFT	NIEHS
M. Mass	Postdoctoral Fellow	LPFT	NIEHS
B. Pai	Former Visiting Fellow	LPFT	NIEHS
V. Steele	Former Staff Fellow	LPFT	NIEHS

POSITION 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 are exposed to carcinogens and/or tumor promoters and cell survival is 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 are assayed for anchorage independent growth in soft agarose and tumorigenicity in nude mice.

MAJOR FINDINGS AND PROPOSED COURSE: We have developed a system to study the cellular mechanism of carcinogenesis that uses normal cells from an environmentally and epidemiologically relevent tissue, respiratory epithelium. The induction of preneoplastic variants of epithelial cells in culture was quantitated on a per cell basis following exposure of rat tracheal epithelial (RTE) cells *in vitro* to the direct acting carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Following treatment of normal RTE cells, large colonies of altered cells exhibiting an enhanced growth potential (enhanced growth variant = EGV) under selective culture conditions were observed while normal RTE cells ceased proliferation after several cell doublings. After further growth in culture, these altered cells acquired the ability to grow in semisolid medium and to produce squamous cell carcinomas when injected into nude mice. The induction of enhanced growth variants of RTE cells by MNNG occurred with a high frequency (>2.6% per colony forming cell). In addition, a linear dose-response curve with a slope of 1 was observed when the logarithm of MNNG-induced transformation frequency was plotted versus the logarithm of MNNG dose. These results are consistent with a one-hit mechanism for induction of preneoplastic variants of RTE cells by MNNG. Similar frequencies and kinetics of induction of preneoplastic variants in other culture systems using diploid cells have been observed suggesting a common mechanism for this early step in carcinogenesis.

The same type of preenoplastic cell variants (EGV) also develop *in vivo*. This was shown by studies in which tracheal transplants were exposed *in vivo* to the polycyclic hydrocarbon dimethylbenzanthracene for periods of 2-4 weeks. After termination of exposure the tracheas were havested, the epithelial lining was removed by protease digestion and dissociated into a single cell suspension, which was then plated in culture (see above). The enhanced growth variants

induced in vivo were similar to those found in cultures exposed to MNNG in vitro. After extensive subculturing (15-20 passages) neoplastic variants developed indicating that EGV are indeed cells with an increased propensity to give rise to neoplastic offspring. Further studies of enhanced growth variants showed that they have nutrient and substratum requirements which clearly distinguish them from untransformed normal cells. To expand these studies concerning the characterization of the phenotypic alterations of the induced in vivo EGV a more convenient in vivo-in vitro model is being developed using carcinogenic nitrosamines, which can be administered systemically. A series of experiments were completed to quantitate the cytotoxic effects of these nitrosamines. Next the transforming effects of different doses of nitrosamine will be investigated.

Studies on the effects of known mouse skin tumor promoters (and inhibitors of promotion) on normal and initiated RTE cells are in progress. The initial objective of these investigations is to determine whether phorbol ester and nonphorbol ester mouse skin tumor promoters affect rat tracheal epithelial cells in culture and to characterize the response particularly with regard to cell growth parameters. It was found that several of the phorbol esters active in the mouse skin promotion assay cause a 6-10-fold increase in colony forming efficiency of primary rat tracheal cells. This effect will be further analyzed. These studies suggest that phorbol esters are able to trigger cells to enter into the cell cycle and to replicate. The effects of tumor promoters in the rat tracheal cell transformation system will be examined to determine if they increase the number of preneoplastic cells following low dose carcinogen treatment and increase the rate of progression of these cells. We recently showed in collaboration with Drs. Terzaghi and Klein-Szanto from Oak Ridge, that the phorbol ester TPA enhances the induction of tracheal carcinomas in vivo. A question which will be addressed in future studies is whether tumor promoters act by inducing new preneoplastic variants or by stimulation of replication and selection of existing variants. In this context we will also examine the effect of antipromoters such as retinoids to determine their effect on the induction of early transformants (EGV) and late transformants such as anchorage independent and neoplastic growth variants. The retinoids are of particular interest in the RTE cell system since they are known to play an important role in the normal differentiation of respiratory tract epithelium. It may thus be possible using retinoids to elucidate the relationship between disruption of cellular differentiation during various phases of carcinogenesis and the emergence of the neoplastic phenotype.

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 often detected at such an advanced stage that prognosis is poor. Our current studies continue to be focused on identifying both early and late cellular alterations during neoplastic progression. By characterizing the various preneoplastic phenotypes and elucidating the mechanisms of progression in epithelial carcinogenesis it is hoped that approaches will be developed to inhibit neoplastic progression and either prevent or delay the development of malignant cell populations.

PUBLICATIONS

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Nettesheim, P,, and Barrett, J.C.: Tracheal epithelial cell transformation: A model system for studies on neoplastic progression. CRC Press, In press, 1983,

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25007-05 LPFT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Deposition of Inhaled Particles and Pathogenesis of Initial Pulmonary Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
A. R. Brody Senior Staff Fellow LPFT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

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 ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inhalation of asbestos and other mineral dusts leads to interstitial pulmonary fibrosis in animals and man. In previous work, we established the initial deposition sites and mechanisms of translocation of inhaled asbestos in rats. Now we demonstrate that a variety of dusts (i.e., chrysotile and crocidolite asbestos, fiberglass, silica, and Mt. St. Helen's Ash) exhibit a similar deposition pattern at the bifurcation of alveolar ducts. Only forty-eight hrs. after a one-hr. exposure to chrysotile asbestos, a significant lesion measurable by ultrastructural morphometry developed at alveolar duct bifurcations. The lesion is characterized by: 1) a thickened epithelium where numerous type I and type II cells contain asbestos, 2) increased numbers of alveolar macrophages, and 3) an increased interstitial compartment consisting of fibroblasts, myofibroblasts and extracellular matrix. The lesion persists at bifurcations for at least one month and exhibits an increase in interstitial volume, comprised mainly of fibroblasts, macrophages and collagen. Interstitial intracellular microcalcifications containing asbestos have been identified at bifurcations as early as one month after a one hr. exposure, and these persist for at least five months post-exposure.

The elemental content of asbestos is believed to play a significant role in its cytotoxic potential. Thus, we have carried out electron microscopic and X-ray analytical studies on chrysotile fibers which have resided in the lungs of rats through the five month period after a one-hr. exposure. We were surprised to learn that fibers in macrophages, epithelial cells, fibroblasts and interstitial connective tissue showed no significant changes in the ratio of magnesium to silicon when compared to fibers which had just been inhaled.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

A. Brody	Senior Staff Fellow	LPFT	NIEHS
L. Hill	Chemist	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: White rats were exposed in inhalation chambers to aerosols of five different mineral dusts: 1) chrysotile asbestos, 15 mg/m^3 for 1 hr.; 2) crocidolite asbestos, 5 mg/m^3 for 1 hr.; 3) fiberglass, 8 mg/m^3 for 1 hr.; 4) alpha-quartz, 110 mg/m^3 for 3 hrs.; and 5) Mt. St. Helen's Ash, 12 mg/m^3 for 1 hr. Mice also were exposed to chrysotile asbestos for 1 hr.

Immediately after exposure (i.e., 4 min 35 sec post-exposure) the animals were anesthetized by an intraperitoneal injection of Nembutal. The lungs were vascularly perfused through the pulmonary artery with 0.9% saline and then a glutaraldehyde-paraformaldehyde combination. The lungs were then excised in toto and submerged in fresh fixative overnight. Tissue blocks (ca. 5 mm^3) were dissected from the left lung and prepared for scanning electron microscopy (SEM). The blocks were further dissected in order to reveal specific anatomic units of the distal lung i.e., terminal bronchioles (TB) and associated alveolar ducts (AD) and alveolar spaces (AS).

In order to quantitate the particle deposition pattern, anatomic units were identified and alveolar duct bifurcations numbered according to increasing distance from the terminal bronchiole (i.e., BF no. 1 is closest to the TB, while BF no. 2 is next distal, etc.). Three regions were then preselected on each bifurcation at low magnification (200X). Subsequently, regions on duct surfaces adjacent (ca. 10-20 μm) to the BF also were pre-selected at low magnification. Finally, pre-selected areas on AD surfaces were chosen at midpoints measured between consecutive bifurcations. All pre-selected areas on the alveolar duct bifurcations and alveolar duct surfaces then were examined at a higher mangification (6600X). Secondary or backscattered electron imaging (BEI) was utilized to count the number of deposited particulates in each FOV.

Conventional ultrastructural morphometry was used to establish tissue area and volumes of first alveolar duct bifurcations were from sham and chrysotile asbestos-exposed rats after 1 hr. of exposure.

Asbestos-induced intracellular microcalcifications were identified by X-ray energy spectrometry in the lungs of rats exposed for 1 hr. to chrysotile asbestos. Similarly, asbestos fibers in macrophages, epithelial cells, fibroblasts and connective tissue were identified by transmission electron microscopy (TEM) and X-ray spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: In rats exposed to aerosols containing chrysotile and crocidolite asbestos, fiberglass, α-quartz, or Mt. St. Helens ash, accumulations of the inhaled particles were found on alveolar duct bifurcations (BF). Scanning electron microscopy suggested that fewer particles had deposited on adjacent alveolar duct (AD) surfaces. Deposition within individual alveolar spaces rarely was observed.

Quantitative data clearly demonstrated a signficantly higher degree of deposition at BF than on adjacent AD surfaces. In all animals studied, the percentage of AD fields of view (FOV) with no particles present was significantly greater than on BF surfaces ($p<0.01$). Accordingly, the percentage of FOV with particle counts greater than 5 (fiberglass and Mt. St. Helens ash) or 10 (chrysotile and crocidolite asbestos and alpha-quartz) was significantly ($p<.01$) higher on BF surfaces compared with those examined on the AD surfaces. This pattern of deposition was consistent at the three levels of bifurcations studied. In addition, there was little evidence of particle deposition on AD surfaces at mid points between BF. The particle deposition pattern in mice exposed to aerosolized chrysotile asbestos was essentially the same as in the rats. The majority of chrysotile fibers accumulated at alveolar duct bifurcations. Significantly fewer fibers were observed on surrounding alveolar duct surfaces.

The biological significance of enhanced particle deposition at alveolar duct bifurcations is not entirely clear. There is evidence suggesting that development of the initial lesions of asbestosis are a result of cellular responses at alveolar duct bifurcations(see below). Reactions such as macrophage accumulation, increased sizes of intersititial and epithelial cell populations and formation of interstitial intracellular microcalcifications all have been documented at alveolar duct bifurcations in asbestos-exposed animals. Development of lesions at bronchiolar-alveolar junctions in humans occupationally exposed to asbestos and silica has been recognized for many years. Even though all levels of the alveolar region are exposed to dust during clearance, disease progression which is initiated at duct bifurcations could be due to increased particle dose from enhanced deposition. Experiments designed to address this hypothesis form the basis for ongoing studies.

Two days after a 1 hr. exposure to chrysotile asbestos the thickness of epithelium and interstitium at bifurcations increased by 85% ($p<0.001$) as determined by ultrastructural morphometry. The total volume of alveolar macrophages on the bifurcations increased nearly 14 fold ($p<0.01$) while that of interstitial macrophages increased 5 fold ($p<0.001$). Statistically significant increases in the volumes of type I cells, type II cells, and interstitial fibroblasts also occurred. The numerical density of alveolar macrophages increased more than 20-fold ($p<0.05$), whereas the numerical density of interstitital macrophages tripled ($p<0.01$). One month after the 1-hr exposoure the numerical density of interstitial macrophages was still increased more than 3 fold ($p<0.05$). The thickness of epithelium and interstitium and the total volumes of type II cells, fibroblasts, and interstitial macrophages were still significantly greater in the

exposed animals. The results indicate that a brief exposure to chrysotile asbestos will cause a rapid influx of macrophages to the region of the first alveolar duct bifurcations and that this is accompanied by local thickening of the epithelium and interstitium, These responses do not fully resolve after one month of recovery. These appear to be the earliest measurable morphologic alterations related to inhalation of asbestos.

One month after the one-hr. exposure to chrysotile asbestos, approximately 50% of the first bifurcations exhibit interstitital intracellular microcalcifications. The calcifications are found in fibroblasts and contain chrysotile fibrils. This lesion persists and has been identified in the lungs of animals as long as 5 months after a one-hr exposure. The asbestos appeared to form a nidus for the intracellular accumulation of calcium and phosphorus as determined by X-ray energy spectrometry. Calcification has long been recognized as a nonspecific response to cell injury. The microcalcifications identified in our model suggest that inhaled chrysotile fibers directly affect interstitial fibroblasts, allowing the influx of calcium and development of the intracellular lesion. The mechanisms of cell injury and formation of intracellular calcifications are the focus of continuing studies in this laboratory.

In earlier studies of inhaled asbestos fibers, an elemental ratio (i.e., Mg:Si) of .72 was established. In our ongoing studies of inhaled fibers through a five month post-exposure period, no significant differences in this elemental ratio have been found in any of the anatomic locations tested. As exposed animals age, these studies will continue. Simultaneously, in vitro studies using chrysotile with varying Mg:Si ratios will be carried out to determine the effects of elemental content on cytotoxic reactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Asbestos clearly is a causative agent of fibrotic and neoplastic lung disease. Millions of individuals are exposed daily to airborne fibers, yet little is known about the basic mechanisms through which asbestos induces disease. Our studies are designed to elucidate the initial pathogenetic events associated with pulmonary asbestosis.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25008-05 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macrophage Accumulation and the Initial Chemotactic Stimulus Activated by Asbestos

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

A. R. Brody Senior Staff Fellow LPFT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Pulmonary Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies have shown that pulmonary macrophages accumulate at sites of asbestos deposition on alveolar surfaces. Pulmonary macrophages are believed to play a central role in the pathogenesis of asbestosis. Accordingly, we have carried out a series of experiments designed to elucidate the initial chemotactic stimulus which attracts macrophages to sites of asbestos deposition where early lesions are known to develop. Four sets of experiments were carried out: 1) Peripheral blood serum from rats was mixed with chrysotile asbestos or zymosan, and a chemotactic factor (probably C5a) for macrophages was activated by both materials (chemotaxis is assayed by counting the numbers of cells migrating through a polycarbonate filter). 2) Proteins concentrated (2.4 mg/ml) from the lung lavage fluids of asbestos-exposed rats showed a significant increase in chemotactic activity compared to concentrates from sham-exposed animals. 3) A chemotactic factor was activated by adding asbestos or zymosan in vitro to protein lavaged from the lungs of normal, untreated rats. 4) C5-deficient mice exposed to asbestos exhibited significantly fewer macrophages on alveolar duct bifurcations compared to normal mice. We hypothesize that inhaled asbestos activates C5a on alveolar surfaces, and this well known chemotactic factor attracts local populations of alveolar macrophages. Biochemical studies to prove this hypothesis are ongoing to separate the lavaged fluids into fractions based on molecular weight (m.w.). Each of the fractions will be tested for chemotactic activity in attempts to support the hypothesis that the major chemotactic stimulus will be in the 15-20,000 m.w. range (i.e., C5a).

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

A. Brody	Senior Staff Fellow	LPFT	NIEHS
D. Warheit	Postdoctoral Fellow	LPFT	NIEHS
L. Hill	Chemist	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: White rats were exposed to an aerosol of chrysotile asbestos for 1 or 5 hrs. Control animals were exposed to air. Forty-eight hrs. after exposure, gentle gravity-dependent lung lavage was carried out with Ca-Mg-free saline. Lavaged macrophages and proteins were separated by centrifugation. Other animals were perfusion-fixed through the trachea for scanning electron microscopy (SEM). Normal mice and congenic C5-deficient mice also were exposed to asbestos, lavaged and perfused as described above. The chemotactic response of alveolar macrophages to lavaged and serum proteins was quantified by counting with light microscopy the number of cells migrating through polycarbonate filters in blind-well chambers. After activation with asbestos or zymosan, lavaged and serum proteins were run through a Sephadex G-100 column to separate suspected chemotactic fractions and ascertain their molecular weight.

MAJOR FINDINGS AND PROPOSED COURSE: Scanning electron microscopy of exposed animals showed that over 90% of first alveolar duct bifurcations (i.e., sites of initial asbestos deposition in rats and mice) exhibited accumulations of significant numbers of macrophages. Bifurcations in sham-exposed animals rarely were associated with even one macrophage. Thus, we formed the hypothesis that macrophages were responding to a chemotactic stimulus produced at the alveolar level. To test this, we incubated normal rat serum with zymosan (a known chemotactic activator) and chrysotile asbestos and found that both materials activated a chemotactic factor (probably C5a) for macrophages. Next, protein concentrated (2.4 mg/ml) from lavage of asbestos-exposed rats was found to promote a significant migratory response in macrophages when compared to protein lavaged from sham-exposed animals, The C5-deficient mice exhibited significantly fewer macrophages on bifurcation surfaces than the C5-normal animals after exposure to asbestos. Finally, in vitro activation of lavaged proteins concentrated from untreated rats resulted in a significant chemotactic response by alveolar macrophages. Proteins separated by the Sephadex column formed 40 fractions, with a clear protein peak for albumin and another for higher molecular weight proteins. Each of these and the lower molecular weight fractions will be tested for chemotactic activity in blind well chambers. The activity of fractions recovered from zymosan-activated serum will be compared with similar molecular weight fractions from asbestos-exposed lavage and in vitro activated lavage fluid. We propose that asbestos fibers impacting on air space surfaces activate serum-derived complement components of the alveolar lining layer to produce C5a, probably by the alternative pathway. This potent chemotactic factor could stimulate the initial accumulation of macrophages. The capacity of these macrophages to attract other cells and influence the development of emphysematous or fibrotic lung responses are under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The progressive pathogenesis of pulmonary emphysema and fibrosis is thought to be mediated, at least in part, by macrophages and neutrophils. These cells migrate into air spaces in response to inhalation of cigarette smoke and organic or inorganic particles such as asbestos, We propose that a chemotactic factor activated on alveolar surfaces plays a significant role in the initial accumulation of pulmonary macrophages at sites of particle deposition in rats and mice.

PUBLICATIONS

Brody, A.R., Hill, L.H., George, G., and Warheit, D.B.: Activation of a chemotactic factor for macrophages on alveolar surfaces. Chest. In Press, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25009-04 LPFT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Membrane Interactions with Chrysotile and Crocidolite Asbestos

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

A. R. Brody Senior Staff LPFT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

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 ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Postively-charged chrysotile asbestos binds to and distorts red blood cell membranes. Using negatively-charged particles and the thiobarbituric acid (TBA) assay for N-acetyl neuraminic (sialic) acid, we have added new data to directly support the hypothesis that hemolysis is mediated through interactions between positively charged asbestos and cell membrane glycoproteins. Five sets of experiments have been completed: 1) Chrysotile and crocidolite asbestos cause over 85% of red blood cells (RBCs) to become distorted only 5-15 min. after treatment. 2) Neuraminidase protects over 85% of the RBCs from the distorting affects of chrysotile, but not crocidoliate asbestos. 3) Treatment of RBCs with chrysotile, but not crocidolite asbestos, caused alterations in the distribution and number of lectin-labeled gold spheres on cell surfaces. 4) The TBA assay showed that asbestos treatment did not cause a release of sialic acid into the medium. Neuraminidase removed over 85% of the sialic acid from normal cells in 3.5 hrs. Chrysotile, but not crocidolite asbestos, prevented the removal of 70% of the sialic acid by neuraminidase. 5) Intracellular analysis of Na^+:K^+ ratios showed that chrysotile, but not crocidolite asbestos, caused significant abnormal ion flux within 5-15 min. after treatment. These experiments strongly support the hypothesis that chrysotile asbestos causes membrane damage through binding to terminal sialic acid residues. Similar studies with pulmonary cells such as macrophages, epithelial cells and fibroblasts will attempt to elucidate the mechanisms through which inhaled asbestos causes cell damage in vivo.

Principal Investigator and All Other Personnel Engaged on the Project:

A. Brody	Senior Staff Fellow	LPFT	NIEHS
L. Hill	Chemist	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Blood drawn by cardiac puncture from white rats was diluted 1:9 in tris-buffered saline. Cells were cleaned by repeated centrifugation in the buffered salt. $1x10^6$ cells were mixed for 5-15 min. in .3 ml of chrysotile or crocidolite fibers suspended at a conc. of 180 ug/ml. Cells were treated similarly with silica crystals as a non-fibrous control particle. Asbestos-treated and untreated control cells were collected on silver-screen filters, fixed, and critical point dried for scanning electron microscopy. To test the effects of neuraminidase treatment on asbestos-red cell interactions, $5x10^6$ RBCs were treated with 1 IU of the enzyme from Vibrio cholera.

The plant lectin Wheat Germ Agglutinin (WGA) binds to n-acetyl neuraminic (sialic) acid and n-acetylglucosamine on cell surfaces. The lectin was conjugated to gold spheres measuring 30 nm in diameter to provide a visible marker of sialic acid distribution on red cell surfaces. This label was applied to cells before and after asbestos treatment.

The thiobarbituric acid assay was used to determine the amount of sialic acid released into the medium or retained with cells which were untreated, asbestos- or neuraminidase treated. Sialic acid was quantified in both the cell and supernatant fractions.

Intracellular ratios of Na:K were determined in treated and untreated RBCs by X-ray energy spectrometry of freeze-dried cells.

MAJOR FINDINGS AND PROPOSED COURSE: Light microscopy and scanning electron microscopy showed that over 85% of untreated red cells exhibited a normal biconcave morphology after four hours in tris-buffered saline. Addition of chrysotile asbestos led to distortion and deformation of 85 ± 5% of the cells within 15 minutes of treatment. Asbestos fibers were intimately associated with red cell membranes, and portions of the membranes were wrapped around fibers. Pretreatment of RBCs with neuraminidase protected over 75% of the cells from the deforming effects of chrysotile asbestos.

Addition of crocidolite asbestos to RBCs also resulted in apparent binding of the fibers and consequent cell distortion. In contrast to the results with chrysotile asbestos, neuraminidase treated cells were protected only slightly from the distorting effects of crocidolite asbestos. In addition, silica crystals caused rapid deformation and hemolysis of red cells.

Scanning electron microscopy (SEM) clearly showed that gold-conjugated wheat germ agglutinin (Au-WGA) was evenly distributed across the surface of normal red blood cells.

Treatment of RBCs with chrysotile asbestos before applying the Au-WGA label significantly altered the normal Au-WGA distribution pattern on distorted cells. The number of Au-WGA labeled sites per unit area of asbestos-reacted red cell surface was reduced to less than 30% of the control level.

Pretreatment of RBCs with crocidolite asbestos and silica crystals produced only small changes in the numbers of Au-WGA labeled sites on distorted red cells. The number of labeled sites on crocidolite-treated cells was ∿85% of control and silica-treated was ∿90% of its control.

Changes in the patterns of Au-WGA labeled sites on RBCs suggested that portions of cell surface glycoproteins had reacted with chrysotile, but not crocidolite asbestos. It was necessary to determine whether or not sialic acid remains associated with RBCs after asbestos treatment, i.e., does asbestos cause release of sialic acid groups into the medium? The data show that ∿95% of the sialic acid remains with both normal and asbestos-treated RBCs. If the sialic acid is bound to chrysotile, but not to crocidolite (as suggested by the Au-WGA labeling data), then neuraminidase should remove significantly more sialic acid from the surface of crocidolite-treated RBCs than from chrysotile-treated cells. Again, the data show that this is the case, i.e., neuraminidase removed over 80% of the sialic acid from normal cells during a 3.5 hr. period. Through the same time period, neuraminidase removed over 75% of the sialic acid from crocidolite-treated RBCs, but only 35% from the chrysotile-treated cells.

X-ray energy spectrometry demonstrated a normal intracellular Na to K ratio of 0.56 ± .17. Red cells analyzed from five to fifteen min. after chrysotile asbestos treatment exhibited a mean ratio of 1.28 ± .25. The crocidolite-treated cells exhibited greater variability in Na:K content, and this group was not significantly different from the untreated or chrysotile-treated groups.

Our findings support the hypothesis that chrysotile asbestos fibers bind to negatively-charged sialic acid residues, causing redistribution of membrane glycoproteins and cell distortion. The nature of crocidolite binding is not clear at this time. Cell deformation, concomitant with sialic acid redistribution and alterations of intracellular Na:K ratio are likely to be integral to the hemolytic process. Inhaled chrysotile asbestos is phagocytized in the lung by a variety of cell types such as macrophages, fibroblasts and epithelial cells. The toxicity which chrysotile asbestos exhibits in macrophages could be attributed to its interactions with phagolysosomal membranes. It seems reasonable to speculate that such cytopathic reactions could be mediated through the positive charge of inhaled chrysotile fibers which bind to negatively charged membrane components as shown here in red blood cells. Studies to address this hypothesis are ongoing with pulmonary cells mentioned above. We have, at this time, successfully labeled with Au-WGA the distribution of sialic acid on macrophage membranes. Similar studies will be carried out with macrophages which are in the process of migrating or phagocytizing a variety of toxic and control particulates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Chrysotile asbestos is the most commonly used naturally occurring fibrous mineral. Crocidolite asbestos is not as prevalent, but both varieties cause fibrotic and neoplastic lung disease in animals and man. These studies are designed to elucidate the mechanisms through which these asbestos varieties injure cell membranes and ultimately cause pulmonary disease.

PUBLICATIONS

Brody, A. R., George, G, and Hill, L. H.: Interactions of chrysotile asbestos with erythrocyte membranes, Environ. Hlth. Perspect. In press, 1983.

Brody, A. R., George, G. and Hill, L. H.: Interactions of chrysotile and crocidolite asbestos with red blood cell membranes: chrysotile binds to sialic acid. Lab. Invest. In press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25012-04 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Lung as an Endocrine Organ Controlling Intravascular Thrombosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

T. E. Eling Research Chemist LPFT NIEHS

COOPERATING UNITS *(If any)*

Dr. L. Marnett, Dr. K. Honn, Wayne State University

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:
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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Using in vitro systems, we have studied the role of peroxidase in control of PGI_2 biosynthesis. Chemicals that stimulate the peroxidase reduce level of hydroperoxides that inhibit PGI_2 biosynthesis, resulting in a stimulation of PGI_2 production. In the future, we intend to study the role of prostaglandin peroxidase in controlling both hydroperoxy fatty acid and possibly leukotriene biosynthesis by pulmonary endothelial cells.

PHS 6040 (Rev. 1/83) GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

T. E. Eling	Research Chemist	LPFT	NIEHS
R. McMillan	Biologist	LPFT	NIEHS

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: We have investigated a mechanism for controlling PGI_2 biosynthesis. The chemical Bayer 6575 is a potent antithrombic and anti-metastatic agent. The bioactivity of Bayer 6575 appears to be mediated by increased PGI_2 synthesis. Preliminary experiments on endothelial cells indicate that Bayer 6575 does not increase PGI_2 by increasing the release of arachidonic acid from endogenous stores. Using an in vitro system, Bayer 6575 and a number of other agents; i.e., DES, estrogen, isoproterenol, etc., stimulated the formation of PGI_2 in a dose dependent manner. The anti-thrombic drug dipyridmole significantly increased PGI_2 biosynthesis, while the vasodilation drug nitroglycerin was moderately effective. These chemicals serve as reducing cofactors for PGS hydroperoxidase which increases PG biosynthesis and decrease levels of the hydroperoxide PGG_2. Increased PGI_2 formation is a result of increasing the formation of the precursor of PGH_2 and protecting PGI_2 synthetase from inactivation by peroxides. The hydroperoxidase component of prostaglandin synthetase thus serves as a means of controlling the level of peroxides in cells and thus controls the arachidonic acid pathways.

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. We intend to examine the peroxidase as means of controlling leukotriene production by endothelial cells.

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 thrombosis.

Pulmonary biosynthesis of PGI_2 (an inhibition of platelet aggregation and thus thrombosis) could be involved in controlling thrombosis. 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 enviornmental agents may have an impact on the state of mechanisms that control the pulmonary function 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

Eling, T., Honn, K., and Marnett, L.: Stimulation of PGI_2 biosynthesis by Nafazatram, Bayer 6575. In Powles, T.J., Bockman, R.S., Honn, K.V., and Ramwell, P. (Eds.): Prostaglandin And Related Lipid, Vol. II. Alan Liss, Inc., New York, 1982, pp. 783-789.

Marnett, L., Siedlek, P., Ods, R., Honn, K., Warnock, R., Tainer, B. and Eling, T.: Stimulation of prostaglandin endopeoxide synthetase and prostacyclin synthetase by the anti-thrombotic and anti-metastatic agent, Nafazatram. J. Biol. Chem. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25014-01 LPFT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Cell-Cell and Cell-Substratum Interactions in Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

A. Jetten	Senior Staff Fellow	LPFT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Cell Biology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	1.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Like the cell population of tracheas in vivo, the population of cells obtained after pronase digestion of rabbit tracheas consists of ciliated, Clara-like, mucous-secreting, and basal cells. It seems likely that the nutritional requirements for cell proliferation and maintenance of these various cells types are different. It appears that the proliferation of tracheal cells in vitro is dependent on the presence of specific growth hormones and nutrients. We have shown that nutrients also play an important role in differentiation of tracheal cells in vitro: depending on whether vitamin A derivatives or serum are present, keratinization is inhibited or stimulated, respectively. Also the substratum provided for trachea cells has an influence on the proliferation, attachment and maintenance of these cells: ciliated cells can be maintained for several weeks on an extracellular matrix from corneal endothelial cells, whereas ciliated cells are absent when cells are plated directly onto plastic culture dishes. Whether cell-cell interactions play a role in this process has to be determined. The objectives of our research is to understand the importance of cell-cell and cell-substratum interactions for cell proliferation, maintenance and differentiation of trachea cells. To approach these objectives we are growing cells on various substrata: different collagens and different extracellular matrices synthesized by various cell lines and determine their effect on attachment, maintenance and differentiation. Moreover, we are identifying the cell surface components and secreted products of these cells (glycoproteins, glycolipids, and proteoglycans) and try to determine the importance of the carbohydrates in cell-cell and cell-substratum interactions. Using this approach we also are attempting to establish specific markers for the various cell types.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

A. Jetten	Senior Staff Fellow	LPFT	NIEHS
J. Rearick	Staff Fellow	LPFT	NIEHS
K. Kim	Visiting Associate	LPFT	NIEHS
M. Porter	Biol. Lab. Technician	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Epithelial cells were isolated from rabbit and hamster tracheas after exposure of tracheal lumen to protease (0.5% at 4°C, overnight). Primary cells were cultured in F12 medium containing epidermal growth factor, insulin, transferrin and pituitary extract. For the identification of cell surface glycoproteins cells are labeled with 2-[^{3}H]-mannose, [^{3}H-]-glucosamine and [^{14}C]-proline. After solubilization of the cells, glycoproteins are examined via polyacrylamide electrophoresis. Extracellular matrix glycoproteins are further examined using specific FITC - conjugated antibodies against laminin, collagen IV and fibronectin. Production of secreted products are examined by labeling cells with [^{35}S]-methionine and [^{3}H]-glucosamine. Mucin production is determined via Sepharose - 4B gel filtration followed by degradation of the void volume by hyaluronidase treatment or β-elimination. Carbohydrates are examined and identified via paper chromatography and HPLC analysis. Production of proteoglycans is determined by labeling cells with [^{3}H]-glucosamine and [^{35}S]-sulfate. Proteoglycans secreted into the medium and associated with the cells are isolated using guanidine HCl extraction and separated via gel and affinity chromatography. The glycans are further characterized and quantitated using several specific glycan-degrading enzymes via thin-layer chromatography. Composition of the carbohydrate chains linked to glycoproteins are examined after chloroform - methanol extraction followed by pronase digestion. The obtained glycopeptide chains are characterized via lectin-affinity chromatography and HPLC analysis.

MAJOR FINDINGS AND PROPOSED COURSE: In the exponential phase primary rabbit tracheal cells in culture divide rapidly and show a rounded-up morphology and little cell-cell contact. After reaching confluency cells appear flatter and show more cell-cell contact. At this time cells also undergo secretion of vesicles or blebbing. When these cells are kept for several days at confluency, cells become squamous and form cornified envelopes. The study of the action of Ca^{++}, serum, and vitamin A analogs on keratinization is in progress. Preliminary results show that serum in a concentration dependent manner stimulate the formation of cornified envelopes dramatically, whereas retinoids block keratinization. Cells were labeled at various stages of growth with [^{3}H]-glucosamine, and cell surface and secreted products, especially the secretion of mucin examined. Secreted material was chromatographed by gel filtration on Sepharose 4B. The void volume peak appeared to be sensitive to hyaluronidase and partially sensitive to β-elimination. Identification of these products is underway and appears to indicate that the void volume material consists of proteoglycans but mucins do not seem to be present in detectable quantities. Identification and quantitation of the synthesis of the different proteoglycans during the various growth phases and squamous differentiation is in progress.

Furthermore, characterization of the glycoproteins and glycolipids and their carbohydrate composition is now starting.

It is clear that attachment of rabbit tracheal cells is affected by different substrata. When cells are plated on plastic tissue culture dishes few ciliated cells attach and are maintained poorly: after a few days in culture no ciliated cells are observed. However, when cells are plated on an extracellular matrix from corneal endothelial cells many ciliated cells attach and are maintained for at least several weeks. Another example showing the importance of the substratum became clear when cells were plated on the extracellular matrix produced by an endodermal cell line Dif 5. On this substratum cells undergo a rapid transition to a squamous phenotype. These results indicate a role for the extracellular matrix in the maintenance and differentiation of tracheal cells. This is now further explored by determining the effects of fibronectin, laminin, collagen Iand IV, and collagen gels on the differentiation of rabbit and hamster tracheal cells especially the effect on keratinization and mucin secretion.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Development of an in vitro cell culture system makes it possible to study the regulation of differentiation on a molecular level. We can determine the mechanisms by which factors such as retinoids, Ca^{2+} and extracellular matrix affect mucin secretion and keratinization. Furthermore, such a system allows the characterization of the carbohydrate structures of the mucins and in what way these are modified by exogeneous and endogeneous factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25017-01 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Proliferation and Its Relation to Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

A. Jetten Senior Staff Fellow LPFT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Cell Biology 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 *(Use standard unreduced type. Do not exceed the space provided.)*

The polyamines putrescine, spermidine and spermine have been linked to various processes of proliferation and differentiation of many cell types. Ornithine decarboxylase (ODC), the enzyme responsible for putrescine formation and probably the rate-limiting step in polyamine biosynthesis, may fulfill an important growth regulatory role. ODC activity is induced by a wide variety of factors. Our research goals are to study the mechanisms by which retinoids, phorbol esters, epidermal growth factor (EGF) and serum control proliferation of airway epithelial cells in culture. We have observed a rapid enhancement in ODC activity by phorbol esters, serum and EGF. This induction of ODC is inhibited by retinoids. We try to understand (1) how phorbol esters and retinoids effect ODC induction and whether their binding proteins or receptors are involved in this action, (2) whether prostaglandin synthesis is responsible for the induction by phorbol esters and the importance of prostaglandins in the control of cell growth, (3) to what extent the ODC activity correlates with proliferation and (4) the role that control of cell proliferation may play in cellular differentiation.

Principal Investigator and All Other Personnel Engaged on the Project:

A. Jetten	Senior Staff Fellow	LPFT	NIEHS
J. Hall	Biol. Lab. Technician	LPFT	NIEHS
J. Shirley	Biol. Lab. Technician	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Established tracheal epithelial cell lines of rat origin were grown in 24 well dishes in modified Waymouth medium containing 5% fetal bovine serum. When reaching confluency cells were treated with various concentrations of phorbol esters and retinoids and after several periods of time, ODC activity was measured. For this, cells were washed several times with phosphate buffered saline and freeze-thawed two times. To the lysed cells an incubation mixture was added containing [^{14}C]-ornithine, dithiothreitol and pyridoxal phosphate. After 60 min incubation at 37°C, reaction was stopped by transferring dishes in ice and perchloric acid was added. The released [^{14}C]-CO_2 was captured on Whatman filters soaked in sodium hydroxide. After addition of hydrofluor radioactivity was determined in a scintillation counter.

MAJOR FINDINGS AND PROPOSED COURSE: Treatment of tracheal epithelial cells with the phorbol ester TPA induces ODC activity reaching an optimum after 4 1/2 hours. Analogs with a high binding affinity to the TPA receptors are potent inducers of the ODC activity whereas analogs that bind poorly don't induce ODC. These results indicate that the TPA receptor may be involved in this action of phorbol esters. Experiments to determine the presence of TPA receptors are in progress.

The induction of ODC by phorbol esters is blocked by cycloheximide suggesting that protein synthesis is required. Preincubation with retinoids inhibits the induction of ODC by phorbol esters. The specificity with which retinoids inhibit ODC correlates well with their specificity to bind to the cytosolic retinoid binding proteins indicating the possible involvement of these binding proteins. In other studies we are trying to correlate the changes in ODC with the effects of phorbol esters and retinoids on cell proliferation. Furthermore, we are trying to determine whether prostaglandins are involved in the regulation of ODC induction. Preliminary results indicate that inhibition of prostaglandin synthesis reduces ODC induction whether this reflects a nonspecific effect or a specific inhibition of prostaglandin synthesis has to be established. Using inhibitors of prostaglandin synthesis we are now trying to correlate changes in prostaglandin production with the effects on ODC and cell growth.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is important to understand the mechanisms by which cell proliferation is controlled. This can lead to a better understanding of the role cell growth control may play in cellular differentiation. Furthermore, the action of retinoids and phorbol esters on ODC can serve as a model to study antagonism between these compounds and their mechanisms of action. This can be especially important for retinoids since they are endogeneous compounds controlling proliferation and differentiation of tracheal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25020-01 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Pulmonary Surfactant

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G. E. R. Hook Research Chemist 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:
1.25	0.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulmonary surfactant is of vital importance to the lungs where it serves to prevent collapse of the alveoli and distal airways at low lung volumes. Pulmonary surfactant is a complex mixture of lipids and proteins which is synthesized in the alveolar Type II cells and stored in the form of cytoplasmic organelles known as lamellar bodies. Secretion of the lamellar bodies into the alveoli results in dispersion of the lamellar bodies and the formation of a film of surface-tension lowering phospholipids over the surface of the alveoli and distal airways of the lungs. Very little is known about the mechanisms through which biosynthesis, storage and secretion of pulmonary surfactant are controlled. Pulmonary surfactant exists in two pools, intracellular and extracellular. By measuring the sizes of these two pools as a function of age, we have sought to establish a relationship between them. Using toxic agents as a means of manipulating pool sizes, we seek to establish the limits of that interpool relationship.

Principal Investigator and All Other Personnel Engaged on the Project:

G. Hook	Research Chemist	LPFT	NIEHS
L. Dethloff	Bio. Lab. Technician	LPFT	NIEHS
L. Gilmore	Biologist	LPFT	NIEHS

POSITION DESCRIPTION

METHODS EMPLOYED: Extracellular lining material is obtained by lavaging the lungs of small animals via the trachea.

MAJOR FINDINGS AND PROPOSED COURSE: Pulmonary surfactant phospholipids may be considered to exist within at least two anatomically distinct pools. The intracellular pool (IP), located within Type II alveolar epithelial cells, is comprised of storage structures called lamellar bodies. The extracellular pool (EP) resides in the alveoli where surfactant prevents alveolar collapse by reducing surface tension at low lung volumes. Methods have been devised for the isolation and quantitation of these two pools. The EP was estimated from the exponential removal of phospholipid from the lungs using bronchoalveolar lavage. The characteristic low density of lamellar bodies was exploited to isolate the IP from lavaged lungs homogenized using a Parr Disruption Bomb at 1700 psi. This method was critical for the estimation of the IP since other methods were considerably less effective in releasing low density phospholipid. The phospholipid compositions of the two pools were similar consisting of at least 80% phosphatidylcholine (PC) (65% of the PC was disaturated). Estimates for the IP and EP sizes in the New Zealand white rabbit are 1.65 ± 0.45 and 2.77 ± 0.49 mg per gram of lung tissue, respectively. The EP/IP increased with age, ranging from 1 at age 1 day to 2.4 at 31 months.

Intratracheal injection of silica (50 mg/rabbit) in saline suspension resulted in marked alterations to both IP and EP. The IP increased 4-fold and the EP 2-fold within one week of the injection. These data indicate that the relationship between IP and EP is not rigidly maintained and that the ratio between the pools may vary considerably. Future studies will focus on the mechanisms through which silica initiates the alterations in sizes of the intracellular and extracellular pools of pulmonary surfactant.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pulmonary surfactant is essential for the maintenance of normal pulmonary functions such as gas exchange. Under conditions where pulmonary surfactant production is impaired or absent the lungs will collapse. The mechanisms through which the surfactant system is regulated and the interaction of these regulatory processes with toxic agents is of fundamental importance in understanding agent-induced pulmonary diseases which involve alterations in quality or quantity of surfactant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80008-09 LPFT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biosynthesis of Prostaglandins (PGs, Hydroxy-Fatty Acids (HFA) and Leukotrienes (LT)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
T. E. Eling Research Chemist LPFT NIEHS

COOPERATING UNITS *(if any)*
Univeristy of North Carolina
Laboratory of Pharmacology, NIEHS

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:
2.2	0.9	1.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pulmonary tissue produces high amounts of prostaglandins (PGs), leukotrienes (LTs), and hydroxy fatty acids (HFA) in response to a number of stimuli or pathological states. These lipids have diverse biological activity. The goal of this study is two-fold, first to develop an understanding of factors with control biosynthesis of these lipids and second to determine their role in secretory and inflammatory processes of the lung.

We have chosen to study the biosynthesis of these lipids in dog tracheal epithelial cells and to determine their role in control of Cl^- and mucus secretion. Dog trachea cells make primary PGE_2 and a number of LTs. LTC_4 and 3 unknown LTs are produced. Cl^- secretion appears to be primarily under control of PGE_2 formation but other data suggest a role from LTs. We intend to characterize further the LTs formed and to relate to Cl^- secretion.

We are also examining the formation of LTs in development of asbestosis. Cultures of rat pulmonary macrophages release LTs in response to asbestos exposure. Macrophage releases primarily LTB_4 but other unknown LTs are released. We intend to further characterize the products released by asbestos from rat macrophages and to explore the possible relationship between macrophages and the development of asbestosis.

Principal Investigator and All Other Personnel Engaged on the Project:

T. Eling	Research Chemist	LPFT	NIEHS
K. Sivarajah	Visiting Associate	LPFT	NIEHS
J. Fouts	Senior Scientist	LP	NIEHS
R. McMillan	Biologist	LPFT	NIEHS
D. Henke	Guest Worker	LPFT	NIEHS
S. Kouzan	Guest Worker	LPFT	NIEHS
R. Boucher	Associate Professor	Dept. Medicine	UNC
A. Brody	Staff Fellow	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG), thromboxane (TX), hydroxy-fatty acid (HFA) and leukotriene (LT) synthetase activities were 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 high pressure liquid chromatography, and estimated by liquid scintillation techniques.

Biosynthesis was studied by adding labeled arachidonic acid to cells isolated from rat, dog and rabbit lung. PGs were seperated 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. Alveolar macrophages were isolated from rat lung and cultured using standard procedures. Intact dog trachea epithelial cells were isolated and Cl^- secretion measured by electrical potential. Mouse skin cells were isolated and separated into purified cell fractions.

MAJOR FINDINGS AND PROPOSED COURSE: We have examined arachidonic acid metabolism in fresh isolated pulmonary cells. 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. We examined arachidonic acid metabolism in cells maintained in culture for 9 days. Surprisingly, the cultures cells produced 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.

We have recently developed an HPLC method for the separation and quantitation of LTs. Using this method we have studied the biosynthesis of these lipids in isolated skin cells and dog trachea cells. Mouse skin epidermal cells convert

AA to PGE_2 and number of unidentified metabolites. These metabolites appear to be LTC_4, LTD_4, and 5-HETE. Further studies are planned to fully characterize these products using UV spectrophotometry and GC-MS.

We have also studied the metabolism of AA in dog tracheal cells and the relationship to CL^- and mucus secretion. Ca^{+2} ionophore stimulates the release of AA and stimulates CL^- secretion. Indomethacin inhibits Cl^- secretion and inhibited PG formation. PGE_2 is the major prostaglandin formed but is only a minor metabolite of AA. The major metabolites appear to be LTC_4 and 3 unknown metabolites. Further studies to identify these metabolites are in progress.

We have also studied the effect of asbestos on the production of LTs by alveolar macrophages. Rat alveolar macrophages make LTC_4 and LTB_4 in response to Ca^{+2} ionophore. Exposure to asbestos rapidly releases AA and produces LTB_4. Further studies will explore the relationship between macrophages release of LT and development of asbestosis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs, LT and HFA have a large diversity of physiological effects. Alterations in these metabolties control cellular events and 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, LT, 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 86: 511-517, 1982.

Ally, A. I., Boucher, R., Knowles, M. R., and Eling, T. E.: Metabolism of prostaglandin endoperoxide by microsomes from human lung parenchyma and comparison with metabolites produced by pig, bovine, mouse and guinea pig. Prostaglandin 24: 578-584, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80023-10 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Nature and Origins of Pulmonary Surfactant

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G. E. R. Hook Research Chemist LPFT NIEHS

COOPERATING UNITS *(if any)*

Molecular Biophysics Work Group, LEB
Department of Pediatrics, Duke University Medical Center

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:
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 *(Use standard unreduced type. Do not exceed the space provided.)*

The alveoli and distal airways of the lungs are lined with an extracellular layer of material which is absolutely essential for the maintenance of normal pulmonary functions such as gas exchange. The most important component of the extracellular lining is a substance called pulmonary surfactant which serves to prevent collapse of the alveoli and distral airways at low lung volumes. Pulmonary surfactant is a complex-mixture of lipids and proteins whose nature and origins form the basis of this research. The objectives of this study are 1) to identify the contribution of each lipid component to the overall function of pulmonary surfactant, 2) to elucidate the role of protein constituents in pulmonary surfactant, 3) to establish the role of complex lipid/protein structures as intermediates in the transformation of secreted lamellar bodies into pulmonary surfactant and 4) to clarify the relationship between the pulmonary surfactant system and the lysosomal enzyme system within the alveolar Type II cell.

Principal Investigator and All Other Personnel Engaged on the Project:

G. Hook	Research Chemist	LPFT	NIEHS
L. Gilmore	Biologist	LPFT	NIEHS
L. Dethloff	Bio. Lab. Technician	LPFT	NIEHS
C. Chignell	Chief	LEB	NIEHS
A. Spock	Prof. of Pediatrics	Duke University	
E. Tombropoulos	Research Chemist	LPFT	NIEHS

PROJECT DESCRIPTION

METHOOS EMPLOYED: Extracellular lining material is obtained by lavaging the lungs of small animals via the trachea. Bronchoalveolar lavage effluents from patients with pulmonary alveolar proteinois 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. Lamellar bodies from the cytoplasm of Type II cells were isolated on discontinuous sucrose density gradients using high speed centrifugation. Electron spin resonance was carried out using a Varian E-109 X-Band Spectrometer. Electron microscopy was carried out using a Philips EM-400 electron microscope.

MAJOR FINDINGS AND PROPOSED COURSE: Pulmonary surfactant removed from the lungs of rabbits,ung pulmonary lavage and isolated with differential centrifugation. This surfactant fraction contained 95% of the total extracellular phospholipids present in lavage effluents and consisted of membranous vesicles, membrane fragments, tubular myelin and secreted lamellar bodies. The fraction was rich in phosphatidylcholine (79.4%) containing 85.2% palmitic acid in the 1-position and 57.4% palmitic acid in the 2-position. Phosphatidylglycerol was the next most abundant phospholipid accounting for 9.4% of the total. Electron spin resonance spectra, using 5-doxylmethylstearate as a probe showed that the extracellular phospholipids of the pulmonary lining were organized into structures which were much more fluid than erythrocyte ghost membranes. The fluidity of phosphatidylcholine isolated from the membranous fraction was similar to that of the fraction itself, indicating that the minor phospholipids had very little influence on the fluidity of the major phospholipid. At physiological temperature, the fluidity of dipalmitoylphosphatidylcholine (DPPC) was relatively low, but could be markedly increased by the presence of 1-palmitoyl-2-oleoylphosphatidylcholine or phosphatidylglycerol (10%). Protein present in the extracellular phospholipid fraction did not effect the fluidity of the fraction. These studies indicate that the unsaturated phosphatidylcholines could play a major role in determining the fluidity of the important surface-tension lowering phospholipids such as DPPC. Since surfactant-associated proteins have been implicated in the function of pulmonary surfactant, future studies will focus on interactions between phospholipids and proteins isolated from the extracellular lining,

Multilamellated structures and tubular myelin, lavaged from the lungs of patients with pulmonary alveolar proteinosis, were examined under the electron microscope and compared with structures lavaged from the lungs of normal humans.

Multilamellated structures, in general, consisted of alternating light and dark lamellae arranged concentrically about membranous vesicles or electron dense bodies. The darker, or more osmiophilic, lamellae consisted of trilaminar membranes and the wider lighter lamellae appeared amorphous. Multilamellated structures were infrequently found in the lungs of normal humans. Tubular myelin from the lungs of patients showed a number of abnormalities including the formation of polygonal tubules and the attainment of very large sizes (up to 70 microns at the widest diameter). Normal tubular myelin consisted of square tubules and rarely exceeded 15 microns at its widest diameter. Both multilamellated structures from the lungs of patients and tubular myelin from the lungs of normal humans contained membranes 85 to 100 A thick and intermembranous amorphous regions which varied from 150 to 300 A in width. A variation of the multilamellated structure was found in which the membranous lamellae were absent although the general organization of the structure was retained, indicating that the amorphous material may be responsible for the overall integrity of the structure. Fused-membrane structures were also examined. Membrane continuities between multilamellated structures and fused-membrane structures indicated that the two structures may be related. These studies suggest that the wide variety of lamellated structures found in the alveoli of patients with pulmonary alveolar proteinois may be derived form tubular myelin. Because amounts of multilamellated structures accumulate in the lungs of patients with pulmonary alveolar proteinois we propose that this disease condition may be a convenient model for the study of processes involved in the formation of tubular myelin. Future studies will focus on the composition of the multilamellated structures and the dissassembly of the structures into their component proteins and lipids.

Recent studies from this laboratory have demonstrated a close relationship between the cytoplasmic storage sites of pulmonary surfactant known as lamellar bodies and pulmonary lysosomes (Hook and Gilmore, 1982). Using lung slices we have further demonstrated that secretion of pulmonary surfactant in the form of lamellar bodies is accompanied by the secretion of lysosomal acid hydrolases such as acid phosphatase, α-mannosidase, and β-N-acetylglucosaminidase. Secretion of these hydrolases could be followed for up to 2 hours before the release stopped. A strickly linear relationship between the amount of α-mannosidase secreted and phospholipid secreted was demonstrated. These studies demonstrate that the association between lysosomal hydrolases and lamellar bodies is not due to the degradation of lamellar body contents by the lysosomal system. Pulmonary surfactant probably arises through an adaptation of the lysosomal enzyme system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Pulmonary surfactant is vital for the maintenance of normal lung functions such as gas exchange since under conditions where synthesis of the material is impaired or absent, the lungs will collapse. Inhaled toxicants such as the oxidant gases (eg. ozone), particulate materials (eg. silica) and chemicals (eg. paraquat) appear to adversely effect production of pulmonary surfactant both qualitatively and quantitatively. The involvement of pulmonary surfactant in the progression and mediation of pulmonary diseases such as respiratory distress of adults and newborn infants and pulmonary alveolar proteinous appears certain.

Unfortunately, the mechanisms which underlie these pulmonary diseases and agent-induced lung damage are not known. Elucidation of the nature and origins of pulmonary surfactant are necessary steps in understanding the disease process.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-07 LPFT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

T. E. Eling Research Chemist LPFT NIEHS

COOPERATING UNITS (if any)

Dr. E. Zeiger and I. Robertson, Laboratory of Molecular Genetics, Drs. Fouts and Anderson, Laboratory of Pharmacology; Drs. C. Harris and H. Autrup, National Cancer Institute

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:
4.1	2.2	1.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

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 system in chemical-induced toxicity or carcinogenesis. We have shown that PGS converts both polycyclic hydrocarbons and aromatic amines to mutagens as measured by bacterial tester systems. Other in vitro studies show the formation of electrophilic metabolites that react with macromolecules. Benzo(a)pyrene-7,8-diol is metabolized to anti-diol epoxide by PGS. We have compared PGS and NADPH-dependent metabolism in hamster trachea and human bronchial explants. In both tissues, stimulation of PGS increases anti-diol epoxide formation. The aromatic amine carcinogen, 2-aminofluorene is metabolized to free radical intermediates by PGS. The stable end product are azo- and nitro-fluorene. Our studies indicate that PGS activates chemicals to ultimate carcinogenic metabolites which may be of importance in initiation of tumors in extra hepatic tissue. Thus PGS is an additional enzyme system to cytochrome P-450 in the metabolism of chemicals.

Principal Investigator and All Other Personnel Engaged on the Project:

T. Eling	Research Chemist	LPFT	NIEHS
J. Boyd	Biol. Lab. Technician	LPFT	NIEHS
R. Mason	Research Chemist	LEB	NIEHS
K. Sivarajah	Visiting Associate	LPFT	NIEHS
D. Josephy	Postdoctoral Fellow	LPFT	NIEHS
G. Reed	Staff Fellow	LPFT	NIEHS
E. Zeiger	Supervisory Microbiologist	LMG	NIEHS
Vacant	Biol. Lab. Technician	LPFT	NIEHS

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 of HPLC. Prostaglandins (PG) and thromboxane (TX) products were also isolated and quantitiated. Tissue cultures were used to examine the interaction of electrophilic PB metabolites produced by PG synthetase with DNA and to determine whether 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 electron spin.resonance (ESR) techniques.

MAJOR FINDINGS AND PROPOSED COURSE: We have 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 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. We have examined the metabolism of BP-7,8-diol in hamster tracheal explants and human bronchial explants. BP-7,8-diol was oxidized to both anti- and syn-diol epoxides. The addition of arachidonic acid stimulated the formation of anti-diol epoxide. The ratio of anti to syn increased from 2:1 to 4:1. Indomethacin reduced the arachidonic acid stimulation. The dependence on the concentration of AA was studied. At a concentration as low as 25 μm, stimulation of metabolism was observed. These results suggest endogenous AA will support oxidation to the anti-diol epoxide. Stimulation of PGS activity also increased the anti-diol epoxide DNA adduct. These studies suggest a role for PGS dependent cooxidation in development of tumor.

We have 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 glutathione 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 as shown by Dr. Zenser was oxidized by PGS to reactive intermediates that bind to DNA but little information is abailable 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 products. 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 by PGS. Metabolism of 2-AF was dependent on arachidonic acid or a hydroperoxide and inhibited by indomethacin. AF inhibited the peroxidatic oxidation of phenylbutazone by PGS hydroperoxidase, suggesting that 2-AF is a reducing co-factor for PGS. The metabolites of 2-AF formed by PGS were isolated and identified by UV-visible spectrophotometry, HPLC, and MS-spectrometry as 2-nitrofluorene and axofluorene. Horseradish peroxide produced the same metabolites while chloroperoxidase produced only nitrosofluorene. Other studies suggest that these stable metabolites are formed from free radicals. 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 it phosphilipid 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 simultaneousl co-oxygenation of xenobiotics can produce toxic metabolites.

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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. Preliminary studies suggested that Clara cells or Clara like cells occur not only in the bronchioles but also in large airways of several species. Our initial studies are therefore aimed at characterizing airway epithelial cells particularly with respect to the occurance of Clara cells.

METHODS EMPLOYED: Ultrastructural and histochemical techniques and dissection of airways from various species.

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. Its percentage of the epithelium various from 17% in the trachea to 51% in the terminal bronchiole. The manuscript describing these findings is in press. In year two, we have addressed 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 compared the nonciliated cell ultrastructure of the tracheas of cats, 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 manuscript

describing these findings has been accepted for publication. The cytochemistry of rabbit tracheal cells at the light microscope level shows that goblet cells have sulfated mucopolysaccharides in their granules and that Clara cell granules do not react with any mucopolysaccharide stain. At the E.M. level, Clara cell granules are of two types: those with a light central core stained positively with dialyzed iron around the periphery, but not high iron diamine, they are probably protein in the core and sialylated glycoconjugates around the rim. The manuscript describing these findings has been submitted. In the current year of this project, we have defined the functions of rabbit Clara cells at all airway level and compared functions at different levels. Three lines of investigation have been followed: 1) complete characterization of secretory product using histo- and cytochemistry, 2) identification of the cytochrome P-450 system within cells of the entire airway tree, and 3) comparison of carbohydrate secretions of rabbit airway epithelium with those of sheep and rhesus monkey. Work on these projects will be complete this year. Prelminary findings indicate marked differences in the structure and carbohydrate content of secretory granules in three species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
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.

PUBLICATIONS

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OAK RIDGE NATIONAL LABORATORY, 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, 1981

CURRENT ANNUAL LEVEL: $144,000

PROJECT DESCRIPTION

OBJECTIVES: This study involves changes in phenotypic expression of antigenic markers on rat tracheal epithelial cells (RTE) as they progress from preneoplastic (nontumorigenic) to neoplastic (tumorigenic) populations in culture. Carcinogen-altered RTE 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 at early cell passages and 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., 1982a) and altered DNA contents (Braslawsky et al., 1982b). The principal objective of this research project is to investigate changes in phenotypic expression of carcinogen-altered RTE cell lines as preneoplastic cultures progress from nontumorigenic 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. 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 radiolabeled antibody binding test (ABT) is of the first type. This test is used to quantitate the amount of tumor antigen on preneoplastic and neoplastic cell populations. The fluorescent antibody test analyzed by flow-cytometric methods is of the second type, which analyzes cell populations for fluorescent yield per cell as well as enumerates the percentage of antigen positive cells. These results are correlated with the relative DNA content of individual cell types. Relative DNA content is determined by incorporation of DNA specific fluorescent dyes and quantitated by flow-cytometric methods. RTE cells from non-carcinogen age-matched controls serve as control populations and are used to establish baseline values for comparison to results obtained using carcinogen-exposed RTE.

MAJOR FINDINGS AND PROPOSED COURSE: The complexity of the syngeneic antitumor response in defining individual antigens that appear on malignant phase cells

has prompted us to produce monospecific reagents to tracheal tumor antigens. Fifteen monoclonal antibodies (MoAb) have been isolated and eleven identified that react with tracheal carcinoma cells, but not with normal cells. Using an epitope-exclusion test, these MoAb have been shown to recognize 6 distinct antigenic determinants (epitopes) that are expressed on all chemically-transformed RTE cells tested but not on non-transformed RTE. Epitopes defined by individual MoAb appear as cell surface antigens and could not be identified with C-type virus protein.

Epitopes were detected on all chemically altered RTE cell lines at both pre-neoplastic and neoplastic cell passages regardless of the carcinogen used for induction. Epitopes were also expressed on cells propagated either in vivo or in vitro and on DMBA induced rat tracheal carcinomas transformed and passaged in vivo (not adapted to culture).

The coordinate changes in DNA content and antigen expression defined by monoclonal antibodies as RTE cultures shift from nontumorigenic to tumorigenic populations in now under study. Results indicate that carcinogen-altered cell populations can be "phenotypically" distinguished and quantitated from non-altered cells in culture by the appearance of neoantigens (defined by monoclonal antibody), and by an apparent change in nuclear DNA content (measured by fluorocytometric techniques). Additionally, for some of the cell lines tested, non-tumorigenic cells can be distinguished from tumorigenic cells by a change in expression in these markers. Longitudinal studies of individual cell lines indicate that cells having the neoplastic phenotype rapidly emerged as a distinct cell type from already altered nontumorigenic populations. To substantiate this hypothesis, it will be mandatory to prove that cells having neoplastic characteristics are not present during the preneoplastic phase. This will be investigated using subclones from preneoplastic cell passages of two cell lines that showed major shifts in both antigen and DNA content during transition from nontumorigenic to tumorigenic cell types, and from one cell lines that showed a change in DNA content but no apparent change in antigen content during transition. Using this approach we will quantitate on a cellular basis the percentage of cells having neoplastic characteristics at successive cell passages before and during conversion to neoplastic populations.

The expression of individual epitopes on non-respiratory tract carcinomas of the rat is variable. Four of the six epitope groups identified appear to recognize "tissue-specific" epitopes. MoAb that defined these 4 epitopes bound exclusively to malignant rat cells of respiratory tract origin. Antigenic determinants in the remaining two epitope groups were additionally expressed on other non-respiratory tract rat carcinomas. Preliminary experiments will be done to determine if these epitopes are selectively expressed on carcinogen-exposed Rauscher leukemia virus (RLV)-infected rat embryo cells. Infection of rat embryo cells with RLV has been shown to enhance chemical transformation and several studies have demonstrated the utility of RLV-infested rat embryo cells as a reliable assay system to screen chemical carcinogens. This assay system is currently under evaluation through the National Toxicology Program.

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 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. Quantitation of antigen-positive phenotypes in mixed populations will also allow study of progenitor-progeny relationships that have evolved as a result of carcinogenic 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 neoplastic transformation of putative carcinogens.

The enhancement by retroviruses of in vitro cell transformation by chemicals is currently under evaluation by the National Toxicology Program. A serious drawback of the assay is that transformation cannot be quantitated on a per cell basis. The identification of monoclonal antibodies that recognize antigen positive transformed cell types would allow for quantitation of transformational activity of putative carcinogenic compounds, provide detailed information on dose-effects and offer defined end-point analysis. At present cultures are only scored as positive or negative. Monoclonal antibody binding would provide additional information to the morphological methods currently used.

PUBLICATIONS

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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

- Hormone regulation of protein synthesis in the prostate, seminal vesicle and and uterus
- Molecular characterization of androgen dependent rat and mouse seminal vesicle genes

B. Hormone Regulation of Embryonic and Fetal Gene Expression

- Normal and glucocorticoid influenced expression of secretory protein genes in developing liver and yolk sac
- Molecular characterization of glucocorticoid responsive genes
- Identification of putative regulatory DNA regions via DNA-mediated gene transfer and *in vitro* hormone-receptor binding

C. Biochemical Basis of Estrogen Action

- Characterization of nuclear events involved in estrogen action in the mouse uterus
- Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites

- Analysis of proteins of the mouse uterus which are involved in the estrogenic response
- Differential uterine cell responsiveness to estrogen

C. Biochemical Basis for Craniofacial Teratogenesis

- Glucocorticoid involvement in prenatal craniofacial development
- Role of adult and embryonic epidermal growth factor (EGF) in prenatal growth and differentiation
- Biochemical basis of TCDD teratogenesis

II. ULTRASTRUCTURAL/CELLULAR STUDIES

A. Ultrastructural Changes as Predictors of Functional Abnormalities

- Correlation of scanning and transmission electron microscopy observations with biochemical, histological, or functional changes in the male and female mouse genital tract as well as cells in culture

B. Toxication/Detoxication of Environmental Chemicals by Target Tissues Related to Reproduction

- Polycyclic hydrocarbon metabolism by rodent testes
- Increased TCDD induction of prostatic aryl hydrocarbon hydroxylase (AHH) following treatment with DNA damaging agents
- Characterization of diethylstilbestrol and steroidal estrogen metabolism and the elucidation of metabolic pathways which produce metabolites of differing biological activities
- Elucidation of the interaction of estrogen metabolism and prostaglandin biosynthesis

C. Toxicology of Early Development

- Chemical effects on preovulatory oocytes and preimplantation embryos

III. CELL/TISSUE/EMBRYO STUDIES

A. Sperm

- Protein analysis of epithelial cells from precaput, caput, corpus and cauda epididymides to identify sperm maturation factors
- Interaction of plant lectins with sperm surface proteins during epididymal maturation
- Monoclonal antibody analysis of sperm surface proteins during maturation and following chemical exposure

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

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
- Morphological and functional characterization of heterologous cultures of testes and Mullerian ducts derived from DES-exposed and unexposed fetal mice
- Morphological characterization of long-term fetal tissue grafts

D. Cell Cultures as Model Systems

- *In vitro* neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally or functionally related chemicals
- *In vitro* growth and differentiation of palatal epithelial cells
- Hormonal control of differentiation and proliferation of seminal vesicle epithelial cells in collagen gel cultures
- Gene expression during embryonal carcinoma cell differentiation *in vitro* as a model for early embryonic development

IV. WHOLE ANIMAL STUDIES

A. Toxicology

- The teratogenicity of glucocorticoids
- Characterization of reproductive tract function (including fertility and carcinogenicity) in male and female mice exposed *in utero* to diethylstilbestrol
- Correlation of improved histopathologic assessment (semi-thin sections) of testicular damage induced by anticancer drugs with sperm counts and fertilizing capacity
- Susceptibility of testicular tissue to early postnatal treatment with antineoplastic agents

B. Data Extrapolation to Man and Risk Estimation

- Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans
- Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons
- Short-term *in vitro* assay for teratogens using human embryonic cells

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 protein synthesis in the prostate, seminal vesicle and uterus: 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 and mouse 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.

B. Hormone Regulation of Embryonic and Fetal Gene Expression

Normal and glucocorticoid influenced expression of secretory protein genes in developing liver and yolk sac: The endoderm cells of the visceral yolk sac are responsible for the synthesis of several proteins found in fetal serum and amniotic fluid, notably alphafetoprotein (69,000 dalton), metallothionein (6,000 dalton) and transferrin (75,000 dalton). Synthesis of these proteins is confined to visceral endoderm cells prior to liver differentiation, when fetal hepatocytes become the second cell type to produce these proteins. Transcription of the alphafetoprotein and metallothionein genes can be regulated by glucocorticoid hormones in neonatal and adult liver. However, it is not known if glucocorticoids can influence expression of these genes in the embryo or fetus. Glucocorticoid effects on transcription of these genes are being studied by assaying for alphafetoprotein and metalliothionein mRNA using cloned cDNA probes. Synthesis of these proteins is being studied by gel electrophoresis of labelled proteins extracted from short term organ culture or from a reticulocyte lysate *in vitro* translation system.

Molecular characterization of glucocorticoid responsive genes: Genes being actively transcribed are thought to differ structurally from inactive genes. Active genes being more sensitive to nuclease cleavage and containing lower levels of the modified base 5-methylcytosine, the relationship between activity of the alphafetoprotein and metallothionein genes and their chromatin structure and base modification are being studied. The natural or chromosomal gene for alphafetoprotein has been purified and characterized by restriction endonuclease mapping and heteroduplex mapping. Various regions of this gene have been subcloned into plasmids and purified for use as hybridization probes and for sequence analysis. The metallothionein gene will be isolated from a λ charon 4A mouse gene library.

Identification of putative regulatory regions via DNA-mediated gene transfer and in vitro hormone-receptor binding: The 5'-flanking DNA sequences of hormone responsive genes are involved in binding of hormone-receptor complexes and RNA

polymerase II. These binding events may regulate qualitatively and quantitatively the transcriptional activity of hormone responsive genes. The 5 -flanking DNA of the AFP gene is being used to study binding of glucocorticoid-receptor complexes in vitro. Sequence analysis of this region coupled with deletion mutagenesis will allow for mapping of putative control regions for the AFP gene. Cultured cells can be transfected with foreign DNA. This DNA can be integrated into chromosomes and expressed by the recipient cell. The 5 -flanking DNA sequences of the AFP gene is being fused with the HSV-thymidine kinase gene and the fusion product introduced into a thymidine kinase deficient mouse cell line which contains glucocorticoid receptors. This system is being used to examine the involvement of specific sequences of the alphafetoprotein gene in response to glucocorticoid in vivo.

C. 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 resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo uterine 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 1-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 isomers, which are possible DES metabolites and which show receptor binding comparable to DES, but were 20-100 times less biologically active. Studies are continuing to determine if the DES compounds have any influence on neuroendocrine activity. Preliminary results indicate that the indenestrol isomers show differences in their ability to suppress LH secretion. Testing of other compounds will be performed to determine if the same degree of biological activity exists at this in vivo estrogenic target site. The ψ-DES exists in E and Z isomeric forms having subtle structural differences; the hormonal activity of the separate isomers was tested to determine which form may be active. Receptor binding, nuclear translocation and tissue response data (growth, DNA synthesis, etc.) indicate both isomers have less activity than DES.

The E-isomer has significantly lower activity than the Z isomer. This difference may be explained by the fact that although the isomers bind similarly, the E-isomer does not translocate receptor to the nucleus as effectively. Studies are continuing with those compounds by investigating their x-ray crystal structure compared to DES to determine if structural and conformational forms of the compounds differ significantly.

Analysis of proteins of the mouse uterus which are involved in the estrogenic response: 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. Non-enzymatic separation of the three uterine tissue compartments have indicated that some of these proteins are unique to one cell type. These gels will be computer analyzed for qualitative and quantitative differences to determine estrogen responsive protein domains which may exist in different uterine cell types. Proteins from the epithelial compartment show significant isoelectric charge trails suggesting glycoprotein structure. A labeled 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 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.

Differential uterine cell responsiveness to estrogens: In order to understand the tissue responsiveness to estrogens in more detail we investigated the mitogenic action in different uterine cell types. Studies indicated that estradiol induces DNA synthesis and mitosis in uterine stroma and epithelium of sexual immature animals; while, mature adult animals show the mitogenic response only in the epithelium. Loss of stromal mitogenesis occurs between day 28-35 of development and coincides with sexual maturity. Experiments using intact and ovariectomized animals indicate this change can be influenced by the ovary. Studies will continue in order to determine and identify what ovarian substances may be involved in this effect. Epidermal growth factor (EGF) receptors are being assessed in uterine tissue to determine if EGF may play a role in the mechanism of estrogen induced uterine mitogenesis. Results indicate specific EGF receptor binding is present in uterine tissue. Further studies to determine the exact cellular localization of these EGF receptors and whether estrogens influence their expression or activity are underway.

D. 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 human embryonic palate. These mesenchymal cells are being examined in cell culture using a variety of parameters, including cell surface glycoproteins and phospholipids to further define the biochemical basis for glucocorticoid induced teratogenicity.

Role of adult and embryonic 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 of 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. TCDD apears to exert its effect by altering the terminal cell differentiation of the palatal medial epithelial cells. Studies are in progress using primary cultures of palatal epithelial cells to further understand this phenomena.

II. ULTRASTRUCTURAL/CELLULAR STUDIES

A. Ultrastructural Changes as Predictors of Functional Abnormalities

Correlation of scanning and transmission electron microscopy observations with biochemical, histological, or functional changes in the male and female mouse genital tract as well as cells in culture: 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. Experiments are underway to characterize by SEM and TEM the fine structure of mouse genital tract cells grown in culture.

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.

Characterization of diethylstilbestrol and steroidal estrogen 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. Moreover metabolism of DES via the peroxidase pathway was demonstrated in isolated organ cultures of fetal genital tissues but not fetal livers. Current studies include similar metabolic approaches to steroidal estrogen and appear to follow pharmacologic principles established for DES. The estrogenic activities of a series of DES metabolites and analogs were determined. Results of these 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 making generalizations to other classes of estrogenic environmental chemicals.

Elucidation of the interaction of estrogen metabolism and prostaglandin biosynthesis: Studies have demonstrated that during oxidative metabolism of estrogens by prostaglandin synthetase, concurrent increases in prostaglandin levels are seen. This provides a link for early effects of estrogens and induction of prostaglandins.

C. Toxicology of Early Development

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 was determined by culturing the embryos in a media containing 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 correlated 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. Mouse conceptuses of pregnancy day 10 can be grown continuously for 48 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 agents such as the steroid triamcinolone.

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 Mullerian 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. These studies are augmented by 2d maps of the developing protein patterns associated with differentiation of these tissues. 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 Mullerian ducts derived from DES-exposed and unexposed fetal mice: A heterologous organ culture system including DES exposed (or unexposed) testes and/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 Müllerian 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.

D. Cell Cultures as Model Systems

In vitro neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally or functionally 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. In the presence of exogenous metabolic activity systems unscheduled DNA synthesis could be induced in these cell cultures by DES and some of its analogs. Perhaps most important is that DES (at transforming doses) was an efficient inducer of aneuploidy in these cells. Moreover, steroidal estrogens also induced transformation; catechol estrogens were more efficient than the parent compound.

In vitro growth and differentiation of palatal epithelial cells: Differentiation of the palatal epithelium is a complex phenomena consisting of mesenchymal interactions and hormonally directed events. In vivo studies of palatal epithelial development are limited. We have developed an in vitro system whereby the palatal epithelia are culturd on extracellular matrix molecules in a serum-free hormone supplemented media. Studies are in progress to examine hormonal regulation of normal development and teratogen-induced abnormal development.

Hormonal control of differentiation and proliferation of seminal vesicle epithelial cells in collagen gel cultures: Cell cultures in collagen gels with both serum containing and serum free media are used to determine the role of steroid hormone and peptide hormones in the differentiation (as determined by expression of the SVS IV gene and morphology) and proliferation of epithelial cells derived from the seminal vesicles of mice.

Gene expression during embryonal carcinoma cell differentiation in vitro as a model for early embryonic development: An embryonal carcinoma cell line F9 derived from a mouse teratocarcinoma (germ cell tumor) can be induced to differentiate into two distinct populations of extraembryonic cell types, parietal and visceral endoderm. F9 monolayers treated with retinoic acid (Vitamin A) and dibutryl cyclic adenosine monophosphate differentiate into an early embryonic cell type parietal endoderm. F9 cells treated with retinoic acid in suspension culture, differentiate into aggregates called embryoid bodies. Embryoid bodies are so-called because they morphologically resemble early mouse embryos at the two-layered stage. Embryoid bodies synthesize large amounts of alphafetoprotein, transferrin and metallothionein which is characteristic of visceral yolk sac endoderm. This cell culture system is being exploited to analyze molecular mechanisms effecting expression of visceral endoderm specific genes.

IV. WHOLE ANIMAL STUDIES

A. Toxicology

The teratogenicity of glucocorticoids: 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. Whole-body autoradiography after administration and labelled glucocorticoids demonstrates localization of glucocorticoid in the developing secondary palatal mesenchymal cells. Immunocytochemical localization of glucocorticoid receptors demonstrates that a high percentage of the palatal mesenchymal cells possess receptors in contrast to a very low percentage observed in the palatal epithelial cells.

Characterization of reproductive tract function (including fertility and carcinogenicity) 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. The oviducts of DES-treated mice were malformed (developmentally arrested) and develop progressive proliferative epithelial lesions, resulting ultimately in oviductal adenocarcinoma. Dysmorphogenesis of the female genital tract at the cellular level resulted in cellular defects in the ovary which contributed to altered gonadal steroid secretion. 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 saline 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 anti-neoplastic 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 and oviductal malformations in female mice two years before comparable reports in woman. Potency ratios during critical developmental periods in animals and humans are used to develop risk estimates and compare model fitness for selected DES-induced genital abnormalities.

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.

Short-term in vitro assay for teratogens using human embryonic cells: A cell line has been established from the mesenchymal cells from a human embryonic palate (HEPM). These cells have been used to invesitage the biochemical and molecular action of several teratogens. In addition, these HEPM cells are sensitive to the growth inhibitory actions of many teratogens including the glucocorticoids. This growth inhibition assay has been used to develop a short-term in vitro assay which is complementary to other short-term assays for detecting possible environmental teratogens in the human.

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 througnout the world to increase the quality intramural programs.

Dr. McLachlan continues to be involved in collaborative research with Dr. Manfred Metzler of the Institute for Pharmacology and Toxicology, University of Würzburg, Germany; Dr. A. Haney, Director, Division of Reproductive Endocrinology, Department of Obstetrics/Gynecology, Duke University Medical Center; and Dr. B. C. Bullock, Department of Collaborative 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 research project with Dr. Indu Parikh and Dr. Bruno Moncharmont, Burroughs-Wellcome Research Labs, on estrogen receptor antibody immunoassay and antibody preparation; localization of estrogen receptor by antibody localization on two dimensional gels.

Dr. Korach is also involved in collaborative research studies with Dr. Jack Gorski at the University of Wisconsin (Madison) on the effect of DES metabolites on rat uterine DNA synthesis and in collaborative research with Dr. Charles Eldridge at Bowman Gray School of Medicine regarding RIA of steroid hormone and luteinizing hormone levels.

Dr. Pratt continues his collaborative studies with Dr. Tom Shepard, University of Washington; Dr. T. Yoneda, University of Osaka, Japan; and Dr. L. Dencker, University of Uppsala, Sweden.

Dr. Andrews is involved in a collaborative project with Dr. Eileen Adamston at the La Jolla Cancer Foundation in La Jolla, California, in which they are studying expression of genes during embryonal carcinoma cell differentiation. He is also collaborating with Dr. Lash Gedama at the University of Calgary in Calgary, Alberta, Canada, in a study of metalliothionein expression in embryonic mice.

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 and with Dr. Jean Lauder of the Anatomy Department to localize glucocorticoid receptors using immunocytochemistry.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer recognition and scientific relevance of current Laboratory programs is the frequence 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 a Workshop sponsored by the World Health Organization's International Program on Chemical Safety. This Workshop on "Methods for the Integrated Evaluation of Risks for Progeny Associated with Prenatal Exposure to Chemicals" was held in Leningrad, USSR. In addition, Dr. Dixon remains a co-organizer of the Target Organ Toxicity Symposia Series; he is presently Editor-In-Chief of the Target Organ Toxicity Monograph 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. In addition, Dr. McLachlan has participated in the CDC program on premature thelarche.

Dr. Pratt has been involved in the Workshop on "Methods for the Integrated Evaluation of Risks for Progeny Associated with Prenatal Exposure to Chemicals" sponsored by the World Health Organization's International Program on Chemical Safety. This Workshop was held in Leningrad, USSR.

Dr. Korach was an invited speaker at a national meeting organized by Dr. Robert Purdy on "Estrogens and Cell Transformation" sponsored by the Southwestern Research Foundation.

Ms. Newbold presented seminars at the University of Würzburg, Germany, and the University of Basle, Switzerland regarding her research with the Transplacental Toxicology group.

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 Fundamental 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, Biology of Reproduction, and Biochemica Biophysica ACTA.

Drs. Korach and McLachlan serve as coeditors on the Target Organ Toxicity Monograph--Endocrine System.

Dr. Pratt is on the Editorial Boards of Differentiation, Teratogenesis, Carcinogenesis and Mutagenesis, Craniofacial Genetics and Developmental Biology, and Environmental Health Perspectives.

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. Dr. McLachlan is on the graduate committees of students at Duke, UNC and North Carolina State and serves as advisor and coadvisor respectively to graduate students at North Carolina State's Department of Zoology and UNC Department of Biochemistry.

Drs. Dixon, McLachlan, Korach, and Pratt have lectured at UNC, Duke, North Carolina State, and/or Burroughs-Wellcome in areas of their research expertise.

Dr. McLachlan participated in the Advanced Reproductive Physiology Course at Duke University and the Mentor Program of the North Carolina School of Science and Math.

Dr. McLachlan also serves as a research advisor to medical students from UNC and Duke as well as Reproductive Endocrine Fellows from Duke University Medical Center.

Dr. Pratt has organized a graduate level course at the University of North Carolina entitled "Developmental Toxicology and Teratology." Dr. Pratt, Dixon, and McLachlan gave lectures in the Reproductive Toxicology curriculum coordinated by Dr. Pratt.

Dr. Pratt is an Adjunct Professor at the University of North Carolina School of Medicine's Department of Anatomy and Cell Biology.

Dr. Andrews organized a course entitled "Molecular Biology" at the Colorado College, Colorado Springs, Colorado.

Dr. Teng is an Adjunct Associate Professor at the North Carolina State University School of Veterinary Medicine, Department of Anatomy, Physiology and Radiology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70010-07 LRDT

PERIOD COVERED

October 1, 1982 to September 1, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Normal and Abnormal Embryonic Development

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert M. Pratt Head, Experimental Teratogenesis Section LRDT NIEHS

COOPERATING UNITS *(if any)*

Department of Pediatrics
University of Washington, Seattle

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Experimental Teratogenesis

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.6	4.2	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term objectives of these ongoing projects are: (1) to culture postimplantation embryos in order to determine the hormone and growth factor requirements for normal development as well as to understand the mechanisms of abnormal development induced by various teratogens including the glucocorticoids; (2) to further develop and refine our short-term screening assay for potential teratogens using the human embryonic palatal mesenchyme cells in culture; (3) to develop culture conditions which will sustain the growth and differentiation of palatal epithelial cells; (4) to understand the mechanisms at a biochemical level by which glucocorticoids and TCDD induce cleft palate and affect differentiation of the palatal epithelial and mesenchymal cells in culture; and (5) to define hormonal regulation of alpha-fetoprotein and metallothionein gene expression in the embryo.

Principal Investigator and All Other Personnel Engaged on the Project:

PI:	R. M. Pratt	Head, Experimental Teratogenesis Section	LRDT	NIEHS
OTHER:	G. K. Andrews	Senior Staff Fellow	LRDT	NIEHS
	R. P. DiAugustine	Research Chemist	LRDT	NIEHS
	E. H. Goulding	Biological Laboratory Technician	LRDT	NIEHS
	R. I. Grove	Staff Fellow	LRDT	NIEHS
	C. S. Kim	IPA	LRDT	NIEHS
	M. M. Russell	Q Student	LRDT	NIEHS
	W. D. Willis	Biologist	LRDT	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) In vitro culture of the rodent embryo is now possible during a major period of organogenesis when the embryo is extremely sensitive to pertubation by environmental agents. Significant progress has been made to determine the appropriate conditions under which to culture these embryos. We have found that the best growth and development of day 10 to 12 mouse embryos is observed using a 50% mixture of certain lots of fetal calf serum along with Waymouth's medium. Studies are in progress to determine what are the critical growth factors, hormones and nutrients that sustain this normal growth and differentiation outside the maternal environment. We plan to selectively remove certain growth factors and hormones using specific antisera after which the suitability of the depleted serum will be tested in whole embryo culture. Various growth factors such as EGF and somatomedin will be added to these cultures in order to ascertain their influence on development. Glucocorticoids are potent teratogens in vivo but little information is available concerning their effort in early postimplantation development. We have found that in culture glucocorticoids exert stage specific effects on embryonic development in that heart malformations are induced between days 8 and 10 of development in culture whereas cleft lip and other craniofacial malformations are observed between days 10 and 12. Studies are in progress to determine the particular cell types in the embryo which are affected using autoradiography, SEM and TEM.

(2) A short-term inexpensive screening assay for potential teratogens is needed to prioritize animal testing for teratogens. We have developed an assay that detects the growth inhibitory properties of many teratogens using the human embryonic palatal mesenchymal cells in culture. This assay along with another complementary assay is currently being validated by the National Toxicology Program for future use. Studies are in progress to further define and delineate the limits of our assay and to develop and analyze other established cell lines from the human embryonic palate.

(3) The growth and differentiation of the epithelial cells in the developing palatal shelf is a little understood phenomenon mainly due to the lack of a suitable in vitro system. We have developed such a system in which the palatal epithelium is enzymatically removed from the mesenchyme and allowed to attach to an extracellular matrix-coated culture dish rich in type I and III collagen and

fibronectin. Optimal growth factor requirements have been determined by culturing the cells in a serum-free hormonally defined medium. We have found that adult mouse epidermal growth factor is a strict requirement for normal growth and differentiation. Studies are in progress to determine whether embryonic growth factors isolated from normal embryos of transformed embryonic cells in culture will substitute for EGF. EGF receptors have been localized to various regions of the epithelium in culture and appear to be especially high in the palatal oral cells. Future studies will be aimed at determining the influence that various hormones, growth factors and teratogens have on growth and differentiation of the isolated palatal epithelial cells in culture.

(4) Glucocorticoids and the dioxin TCDD are extremely interesting cleft palate teratogens. Both appear to cause this anomaly by a mechanism which is dependent upon binding to a cytosolic receptor followed by translocation to the nucleus and altered mRNA synthesis. Our results strongly suggest that glucocorticoids exert their effect mainly on the proliferation of the palatal mesenchymal cells whereas TCDD exerts its effect on the differentiation of the palatal epithelial cells. We have found that injected labeled glucocorticoids concentrate in the palatal mesenchyme and TCDD in the epithelium. Furthermore, immunocytochemical localization of glucocorticoid receptors in the developing embryo demonstrates a predominant localization in the palatal mesenchyme. Glucocorticoids appear to affect the palatal mesenchyme cells by inhibiting their growth and preventing contact of the apposing palatal shelves. Our studies suggest that this growth inhibition, which is presumably the major effect of glucocorticoids responsible for cleft palate, is due to some mechanism other than the ability of glucocorticoids to inhibit prostaglandin synthesis. Our studies show that dexamethasone produces alterations in the synthesis and degradation of a plasma membrane phospholipid thought to be involved in the mechanism of action of certain polypeptide growth factors. We have demonstrated that a causal relationship may exist between the dexamethasone-induced alteration in phospholipid turnover and inhibition of palatal mesenchyme cell proliferation. Further investigation is being conducted to determine the mechanism by which DEX alters phospholipid turnover.

(5) The alpha-fetoprotein (AFP) gene is expressed only in visceral yolk sac endoderm and liver during embryonic and fetal development of mice. This finding indicates that expression of the AFP gene can provide a useful gene marker for studies of early development in mice. Furthermore, the metallothionein (MT) gene is expressed in fetal liver and also in visceral yolk sac endoderm. Expression of the MT gene in fetal liver has been previously documented; however, expression of the MT gene in visceral yolk sac is a new finding. Although their role in embryonic and fetal development has not been defined, MTs have been implicated in zinc and copper metabolism and have been shown to bind cadmium which has known teratogenic effects.

The embryonal carcinoma system provides an interesting in vitro model with which to analyze events during early embryonic development. Our data suggests that cells which closely resemble visceral yolk sac endoderm are able to differentiate from the embryonal body in the presence of retinoic acid. There is a correlation between expression and lack of 5-methylcytosine residues in the AFP gene. These

data indicate that tissue specific patterns of gene expression may be modulated by changes in patterns of base modification within genes.

The proposed course of this work involves further analysis of the tissue specificity and ontogeny of expression of the AFP gene and particularly the MT gene during embryonic development. This will require measuring specific mRNA levels in various developing embryonic tissues. The effects of glucocorticoids on expression of these genes (mRNAs) in yolk sac and liver will be assessed and correlated with changes in glucocorticoid-receptors, chromatin structure and cytosine methylation patterns within these genes. Further studies will be directed toward analysis of AFP and MT gene expression in developing embryonal carcinoma cells. Development of these cells can be altered by retinoic acid (vitamin A) and by cyclic-adenosine monophosphate.

In addition to the studies described above, in vitro experiments will be conducted which attempt to elucidate the DNA sequence(s) within the AFP gene which interact with glucocorticoid-receptor complexes. These experiments will involve binding cloned AFP gene fragments with hormone-receptor complexes. Further studies will utilize DNA-mediated gene transfer to assess the expression of cloned AFP gene fragments introduced into culture cells.

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. Human embryonic cells would also be useful in predicting the toxic potential of environmental agents and in understanding the biological mechanism by which chemicals may disrupt early development and produce birth defects.

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 highly 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.

Cell specific regulation of gene expression is thought to be the major mechanism by which differentiation occurs. Thus, understanding molecular events which regulate embryonic and fetal gene expression may provide clues as to the mechanisms of both normal and abnormal development. The teratogenic effects of glucocorticoids as well as heavy metals are well documented; however, little is known about the molecular mechanism of action of these substances in the developing embryo and fetus. Studies in progress will attempt to elucidate molecular events involved in regulation of the AFP and MT genes in fetal and embryonic mice. These studies will be valuable in understanding both normal and abnormal development.

PUBLICATIONS

Brown, N.A., Shull, G., Kao, J., Goulding, E.H., and Fabro, S.: Teratogenicity and lethality of hydantoin derivatives in the mouse. Tox. Appl. Pharm. 64: 271-288, 1982.

Fabro, S., Shull, 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.

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. 2: 313-318, 1982.

Schmid, B.P., Goulding, E.H., Kitchin, K., and Sanyal M.K.: Assessment of the teratogenic potential of acrolein and cyclophosphamide in a rat embryo culture system. Toxicology 22: 235-243, 1982.

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Petropoulos, C., Andrews, G., Tamaoki, T., and Nelson, F.: α-Fetoprotein and albumin mRNA levels in lever regeneration and carcinogenesis. J. Biol. Chem. 258(8): 4901-4906, 1983.

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Koropatrick, J., Andrews, G.K., Duerksen, J.D., Varshney, U., and Gedamu, L.: Mouse hepatic metallothionein-I gene cleavage by micrococcal nuclease is enhanced after induction by cadmium. Nucl. Acids Res. (In press).

Pratt, R.M.: Hormones, growth factors and their receptors in normal and abnormal development. In Kalter, H. (Ed.): Issues and Reviews in Teratology. New York, Plenum Press, Vol. 2 (In press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70045-07 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanism of Androgen Mediated Gene Expression in Male Sex Organs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Stephen E. Harris Senior Staff Fellow LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Toxicology

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. In order to study the androgen regulated SVS IV and V genes at molecular level, we have transferred recombinant plasmids which contained the entire SVS IV and pSV2-gpt genes into rat Dunning adenocarcinoma cells (R3327G8A). The accumulation of RNA transcribed from the transfected gene is now under investigation.

Principal Investigator and All Other 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. T. Teng	Expert	LRDT	NIEHS
	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS

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 EcoRI 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. R3327G8A cells were maintained in DMEM supplemental with 10% horse serum. The cells were transfected with recombinant plasmids by the calcium phosphate procedure. Selection for transformants containing the Ecoli gpt gene was then performed in the presence of xanthine and mycophenolic acid.

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. We have found that the region around -100 to -150 bp was differentially susceptible to the S_1-nuclease. DNase I hypersensitive sites of SVS IV gene were present in seminal vesicle cells in which the gene is expressed and were devoid in liver cells in which the gene is repressed.

A 5.2kb BamHI fragment was isolated from the plasmid pSV63 which contained the first and second exon of the SVS IV plus 4kb of 5'-flanking sequences. This fragment was inserted into the BamHI site of vector pSV2-gpt. The resulting plasmids containing SVS IV and Ecoli gpt gene were selected. The recombinant plasmid was transfected into R332768A cells which contained androgen receptors. Our preliminary results showed three of the transformants contained 3 to 5 copies of the SVS IV gene.

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., and 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.

Mansson, P.E., Sugino, A., and Harris, S.E.: Use of a cloned double stranded cDNA coding for major androgen dependent protein in rat seminal vesicle secretion: The effect of testosterone in gene expression. Nucleic Acid Res. 9: 935-946, 1981.

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Teng, C.T. and Harris, S.E.: The seminal vesicle secretion IV gene: Detection of S_1 nuclease-sensitive sites in supercoiled plasmid pSVS 33. DNA 2: 103-109, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70047-07 LRDT

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Prostate as a Model System to Study normal and Abnormal Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Donald B. Carter Senior Staff Fellow LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. 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 were investigated. Several tumor lines from human prostate were investigated for the content of RNA sequences homologous to a battery of oncogene probes. There appear to be no human prostate RNA sequences having homology with the 5 oncogene probes utilized in this study.

This project was terminated in December 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70060-10 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Development and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John A. McLachlan Head, Transplacental Toxicology Section LRDT 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

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The long-range goals of this project are: (1) to evaluate the effects of prenatal exposure to hormonally active 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 and cell culture systems to study the effects of hormonally active environmental chemicals on the development of the fetal reproductive tract in vitro; (8) to study at the cellular and molecular levels alterations in genital tract differentiation induced by hormonally active compounds; and (9) to evaluate the above animal models as predictors of human response. Special attention is given to diethylstilbestrol (DES).

Principal Investigator and All Other 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
	Y. Tomooka	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
	R. Mason	Research Chemist	LB	NIEHS
	R. P. DiAugustine	Research Chemist	LRDT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Pharmacological, molecular, biochemical, physiological, and morphological procedures were used which included one and two dimensional gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, chromatography, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts, and cell cultures of stromal or epithelial target cells were studied.

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.

The control of cell differentiation and proliferation in the genital tract is obviously a central issue to our studies. This will be further studied utilizing mesenchymal and epithelial cell cultures. Moreover, the role of genital tract derived growth factors, including epidermal growth factor (EGF), in the proliferation and development of the uterus will be determined. Alteration of these processes by early treatment with hormones will add insight to their importance.

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. Gene probes for Wolffian duct function and Müllerian duct function will be established and used to study the cell-cell interactions necessary to normal genital tract development. 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. Seminal vesicle epithelial cells will be established in cell culture in defined media and response to hormones studied using genetic techniques and morphology. This model system will be used to study the underlying molecular and biochemical alterations which accompany aberrant differentiation.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g. Z,Z-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. These findings are consistent with peroxidase mediated metabolism in the fetal target tissue. Moreover, a new metabolite monomethylated DES suggests another important pathway for DES metabolism which is observed only in target tissue (*in vivo* or *in vitro*). 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, Z,Z-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 Z,Z-dienestrol was demonstrated. Further studies suggest that metabolism of DES by these cells is mediated, at least in part, by cooxidation 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. Additional studies using steroidal estrogens and their catechol derivatives have demonstrated that the pharmacological principles established for neoplastic cell transformation and drug metabolism pertinent for DES appear to apply to this class of "natural" estrogens. Studies using x-ray crystallography as well as computational models will be used to further explore the structural basis for estrogen induced cell dysmorphogenesis.

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. Currently, a single gene product (70K/5.8 pI) appears to be consistently and permanently deleted in dysmorphogenic genital tracts in DES treated mice. 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. A new model in which a progressive proliferative lesion of the oviduct leading ultimately to oviductal adenocarcinoma is being used to study control of Müllerian-derived epithelial proliferation and differentiation. This model and the dysmorphogenic dysfunctional ovarian model are helping solve the role of tissue interactions (Wolffian/Müllerian) during genital tract development. Finally mathematical models will be developed to test the fit of laboratory animal data in risk assessment for humans.

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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DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70065-07 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Kenneth S. Korach Research Endocrinologist LRDT NIEHS

COOPERATING UNITS *(if any)*

University of Wurzburg
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LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Transplacental Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are: (1) to determine whether DES is metabolized to biologically and hormonally active metabolites; (2) to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins particularly alpha-fetoprotein; (3) to investigate some of the biochemical mechanisms which contribute to effects of prenatal exposure of mice to hormonally active environmental chemicals; (4) to investigate the mechanism of uterine hormonal responsiveness by assessing the nucleic acid and protein synthetic activity; (5) to determine the role of growth factors in hormonal stimulation and to define the molecular loci of transplacental toxicity using structure-function relationships of different environmental chemicals; and (6) 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 and toxic nature of hormonally active environmental chemicals will be studied in vivo.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

PI:	K. S. Korach	Research Endocrinologist	LRDT	NIEHS
OTHER:	J. A. McLachlan	Head, Transplacental Toxicology Section	LRDT	NIEHS
	L. Levy	Research Chemist	LEC	NIEHS
	R. P. DiAugustine	Research Chemist	LRDT	NIEHS

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 quantification 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 month of age there is no pattern of receptor differences. By 2-4 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. Biochemical characterization of the receptor in the DES group showed similar molecular properties and nuclear translocation capacity as controls. 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. Uterine progesterone receptor is an estrogen specific response which can be induced in ovx/adx animals but not in the DES treated group. In order to establish how early in development the receptor system is present, measurements of 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 by comparing the autoradiographic pattern of different steroid hormones. 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. Non-enzymatic separation of the three uterine tissue compartments have indicated that some of these proteins are unique to one cell type. These gels will be computer analyzed for qualitative and quantitative differences to determine estrogen responsive protein domains which may exist in different uterine cell types. Proteins from the epithelial compartment show significant isoelectric charge trails suggesting glycoprotein structure. A labeled 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. Certain uterine proteins on the gels are being identified by antibody localization. This is accomplished by electroblotting the proteins from the two dimensional gels onto nitrocellulose sheets for analysis by immunoprecipitation. Attempts will also be made to produce a poly (A^+) mRNA preparation and library to study the mechanism of their synthesis.

In order to understand the tissue responsiveness to estrogens in more detail we investigated the mitogenic action in different uterine cell types. Studies indicated that estradiol induces DNA synthesis and mitosis in uterine stroma and epithelium of sexual immature animals; while, mature adult animals show the mitogenic response only in the epithelium. Loss of stromal mitogenesis occurs between day 28-35 of development and coincides with sexual maturity. Experiments using intact and ovariectomized animals indicate this change can be influenced by the ovary. Studies will continue in order to determine and identify what ovarian substances may be involved in this effect. Epidermal growth factor (EGF) receptors are being assessed in uterine tissue to determine if EGF may play a role in the mechanism of estrogen uterine mitogenesis. Early results indicate specific EGF receptor binding is present in uterine tissue. Further studies to determine the exact cellular localization of these EGF receptors and whether estrogens influence their expression or activity are underway.

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 uterine 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 isomers, which are possible DES metabolites and which show receptor binding comparable to DES, but were 20-100 times less biologically active. Studies are continuing to determine if the DES compounds have any influence on neuroendocrine activity. Preliminary results indicate that the indenestrol isomers show differences in their ability to suppress LH secretion. Testing of other compounds will be performed to determine if the same degree of biological activity exists at this in vivo estrogenic target site. The ψ-DES exists in E and Z isomeric forms having subtle structural differences; the hormonal activity of the separate isomers was tested to determine which form may be active. Receptor binding, nuclear translocation and tissue response data (growth, DNA synthesis, etc) indicate both isomers have less activity than DES. The E-isomer has significantly lower activity than the Z isomer. This difference may be explained by the fact that although the isomers bind similarly, the E-isomer does not translocate receptor to the nucleus as effectively. Studies are continuing with those compounds by investigating their x-ray crystal structure compared to DES, to determine if structural and conformational forms of the compounds differ significantly.

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 hydroxylated 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 investigated. Preliminary studies indicate this compound interacts with receptor binding sites in a biphasic mechanism and that this complex can translocate to uterine cell nuclei. The derivative also appears to be very effective at stimulating uterine growth and DNA synthesis. 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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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70069-01 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Peptide Growth Factors in Reproduction and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard P. DiAugustine Research Chemist LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Transplacental Toxicology

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Epidermal growth factor (EGF) is a polypeptide capable of stimulating proliferation of a wide range of cell types in vitro. In one of the present studies, EGF extracted from the submaxillary gland of mice was found to exist in multiple forms which can be isolated by high-performance liquid chromatography. Compositional differences, so far, cannot be demonstrated between α- and β-EGF, the two major forms. Neither of the purified forms convert to the other after isolation or exposure to urea. Administration of ^{125}I-α-EGF intravenously to pregnant mice revealed rapid clearance and degradation of the labeled peptide but no evidence for placental transfer of the intact peptide. Gel chromatography of extracts of embryos from 13 day pregnant CD-1 mice revealed that most of the material inhibiting ^{125}I-EGF binding to liver membranes was associated with peptides that did not co-elute with EGF. In another study, EGF is being examined as a putative mediator of estrogen-induced uterine epithelial cell proliferation. Uterine tissue will be studied for binding of ^{125}I-EGF to membrane preparations, autoradiographic localization of receptors, immunolocalization of EGF, and histologic changes following direct introduction of EGF into the uterine cavity.

Principal Investigator and All Other Personnel Engaged on the Project:

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	O. Hernandez	Senior Staff Fellow	LEC	NIEHS
	M. Walker	Chemist	LEC	NIEHS
	J. A. McLachlan	Head, Transplacental Toxicology Section	LRDT	NIEHS
	K. S. Korach	Research Endocrinologist	LRDT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparation of Epidermal Growth Factor: Mouse epidermal growth factor (EGF) was obtained commercially from several sources and further purified by high-performance liquid chromotography (HPLC). The HPLC system consisted of two μBondapak C_{18} columns in series; elution was carried out at 22° with 10 mM diethylenetriamine phosphate, pH 7.0 and 25% (v/v) acetonitrile. Fractions obtained were monitored for u.v. absorbance (280 nm) and competitive binding activity with ^{125}I-EGF and purified rat liver plasma membranes. ^{125}I-EGF was prepared by the chloramine T method and purified over a Sephadex G-50 column. Purified receptor-competing material was analyzed by amino acid analysis and with selected protein/peptide reagents in an effort to determine compositional, structural and physicochemical properties.

Extraction of EGF-Related Peptides from Mouse Embryos or Maloney-Sarcoma Virus Transformed 3T3-B-11-1C cells: Embryos collected from pregnant CD-1 mice were rinsed in chilled phosphate-buffered saline (PBS), frozen in dry ice, lyophilized and stored at -20°C. The pulverized dried embryos were extracted in 1N formic acid at 70°, neutralized with NaOH and dialyzed. The non-dialyzable material (> Mr ∿ 1000) was centrifuged and the supernatant lyophilized. The resulting powder is stored at -20°. The powder was assayed for EGF-receptor binding activity and chromatographed on a P-10 column equilibrated with 1M acetic acid. Fractions are lyophilized and assayed for A_{280} nM and inhibition of ^{125}I-EGF binding to rat liver plasma membranes.

The conditioned media from MsV- transformed 3T3-B-11-1C cells is clarified by low-speed centrifugation and then subjected to continuous-flow hollow-fiber filtration; the same is then dialyzed against 0.1 M acetic acid and lyophilized. The lyophilized material will be chromatographed in the same manner as described above for extracts of mouse embryos.

Intravenous Injection of ^{125}-EGF: In those experiments where we assessed the potential for placental transfer of blood-borne ^{125}I-EGF, each pregnant mouse was given a single intravenous injection (30-50 μl) of ^{125}I-EGF (0.5 - 1.0 x 10^6 cpm) into the tail vein by using a 30 gauge dental needle attached to a microsyringe by a fine-bore polyethylene tubing. Blood was obtained by decapitation with EDTA (1 μg/ml) as the anticoagulant. Placentas and whole embryos were resected, rinsed in chilled PBS, and counted in a gamma counter.

MAJOR FINDINGS AND PROPOSED COURSE: Multiple Forms of EGF: HPLC of commercial preparations of either "culture grade" or "receptor grade" yielded three distinct u.v.-absorbing peaks capable of competing with ^{125}I-EGF (receptor grade) for specific binding sites on liver plasma membranes. The peaks were designated α, β, and γ EGF and corresponded approximately to 48, 22, and 4% of the total u.v. absorbing material; u.v.-absorbance was directly proportional to the competing activity. All three peaks demonstrated receptor competing activity when ^{125}I-α-EGF was used as the radio-ligand. Thus, preparations used by most investigators represent a mixture of multiple forms of EGF and a small number of peptides of unknown chemical and biological properties. The present HPLC system provides a convenient and rapid method for providing homogeneous forms of EGF for biological and analytical study.

Our preliminary amino acid analyses of α- and β-EGF suggest that these peptides have the equivalent amino acids originally reported for EGF isolated from male mouse submaxillary glands. It is possible that the different forms occurred through side-chain modification, partial cleavage of the peptide backbone, or formation of oligomers. Determination of the NH_2 - terminal and COOH - terminal amino acids is now in progress.

We are also investigating whether these forms differ by the degree of oxidation of the one methionine residue (amino acid 21). Oxidation of α- and β- forms with chloramine T yielded more polar peptides (Met-SO_2 EGF) that eluted near the void volume. Treatment with CNBr/formic acid essentially destroyed the peptides eluting at the retention of α- and β-EGF, indicating that the methionine in both peptides was not oxidized. Met-SO_2 does not undergo cleavage with CNBr.

The potential for interconversion of α and β forms is being explored after treatment of the peptides with various reducing agents and other denaturants. We are interested to know whether reduction (with β-mercaptoethanol) and alkylation (iodoacetamide) of α- and β-EGF yield modified peptides with the same retention times by HPLC. This would indicate that conformation, not composition, is the major determinant of multiple forms of EGF. Stored preparation of mixtures of α-EGF or β-EGF revealed no evidence for interconversion of α and β forms. Preparations mixed with 7-8 M urea for over 10 hrs. also revealed no interconversion after direct application of the mixture to HPLC columns.

Ultimately, we should like to understand whether the α and β forms exist in vivo and, if so, explore their relative significance in the storage, secretion, and mechanism of action of EGF.

Assessment of Placental Transfer of Blood-Borne EGF: Following intravenous injection of ^{125}I-EGF (receptor grade) or ^{125}I-α-EGF into mice 10-17 days pregnant, the labeled peptide was rapidly cleared from the blood (half-life < 2 min). Column chromatography (Sephadex G-50 or G-15) of samples of plasma collected at various time intervals after injection revealed that by 5 min very little intact ^{125}I-EGF was present. Labeled fragments were not observed eluting between intact ^{125}-EGF and the column volume (V_T) on Sephadex G-50. Plasma samples taken

between 5 and 120 min after injection yielded radioactivity near the void (V_0) and elution volume of $Na^{125}I$. We are currently investigating the identity of materials associated with the labeled peaks, especially for free ^{125}I or ^{125}I-monoiodotyrosine.

During the period where intact ^{125}I-EGF was present in the plasma, virtually no radioactivity was detected in embryos resected from mice pregnant for 10, 13, or 17 days; significant radioactivity, however, was present in the whole placenta. Uptake of radioactivity into embryos occurred predominantly after 15 min, when most of the plasma ^{125}I eluted in the total column volume. No significant difference was observed in the disposition between ^{125}I (receptor grade)-EGF and ^{125}I (HPLC-purified)-α-EGF.

Excluding a delayed placental transfer of intact ^{125}I-EGF, we may assume from the data that EGF does not enter fetal cord blood. This suggests that maternal blood-borne EGF, per se, does not have a significant role in embryonic development. As with plasma samples, we are currently extracting placentas and embryos at selected periods following administration of ^{125}I-EGF in order to assess the chemical identity of materials contributing to the radioactivity in these tissues.

Identification of EGF-Related Peptides in Mouse Embryonic Tissues: Some investigators have postulated that since EGF can stimulate the growth of cultured embryonic cells and specifically bind to membranes of embryonic cells, endogenous EGF-like ligand(s) may be present in normal embryonic tissues. This factor could serve, as has been suggested by others, to stimulate the rapid proliferation observed in embryonic tissues. To investigate this possibility, formic acid extracts of embryos obtained from 13 day pregnant CD-1 mice were examined for competition with ^{125}I-EGF for specific binding to liver plasma membranes. The displacement of the radioligand appeared parallel to that produced by mouse EGF. Approximately 1-1.5 ng EGF equivalents was present per mg of the crude extract. Since EGF is known to bind anomalously to polyacrylamide columns under acidic conditions, the extract was chromatographed on P-10 columns equilibrated with 1 M acetic acid and receptor-competing activity measured in the lyophilized fractions. Most of the competing activity of the extracts of mouse embryos was found in material eluting slightly earlier than insulin (Mr ~ 6600). A peak of activity was found that co-eluted with ^{125}I-EGF. Since extracts of mouse embryos were previously shown to contain a transformation growth factor (TGF) similar to that identified in virus-transformed mouse embryonic fibroblasts, we intend to compare the biological and chemical properties of the major peak material we obtained with that found in extracts of the conditioned medium of the MsV-transformed mouse 3T3-B11-1C cells. These substances will be examined for their capacity to alter the growth and appearance of cultured mouse embryonic palatal mesenchymal cells and oral and nasal epithelial cells derived from mouse embryos at gestational age of 13-1/2 days. It is conceivable that TGFs, which are known to bind EGF receptors and a separate specific receptor population, contribute to the normal rapid proliferation of cells in developing embryos.

Does EGF or Other Peptide Growth Factor Mediate Estrogen-Induced Uterine Epithelial Hyperplasia? The mechanism by which estrogens stimulate epithelial cell proliferation in the uterus is not presently known. Studies with tumor cell lines from estrogen-responsive organs, have shown that tumor growth is stimulated in vivo by estrogens in the host animal, but no direct estrogen-induced mitogenic effect can be shown in culture.

One possibility that may explain this discordance is that the cultured cells lose their estrogen receptors. However, cell lines such as the clonal pituitary cell line (GH 3/C 14) apparently contain estrogen receptors (K_d = 0.25 nM) but still are not stimulated to proliferate in vitro or alter the cell-cycle distribution in the presence of physiological concentrations of estrogen; translocation of the cytoplasmic receptor to the nucleus also appeared to remain intact. The serum from estrogen-treated ovariectomized animals also failed to stimulate mitosis.

To explain this paradox, it has been postulated that estrogens may induce growth factors in selected organs that are the true mediators of estrogen-promoted proliferation of tumors and selected cell populations in the normal mammary gland, anterior pituitary, kidney and uterus. In agreement with this notion, a protein extracted from the rat uterus stimulated the growth of MTW 9/PL mammary tumor cells in vitro. The relative levels of this apparent growth factor(s) was reported by Sirbasku and colleagues to be stimulated by treatment with estrogen; ovariectomy reduced the specific activity of the extracts. The data suggest that estrogen may trigger synthesis or increased availability of the growth factor in the uterus.

Since EGF is known to stimulate proliferation of a wide range of cell types in vitro, in particular, primary cultures of the mammary epithelium, this laboratory has recently begun a collaborative effort to explore a putative role for EGF in the estrogen-induced proliferation of mouse uterine epithelial cells. In this study, we intend to examine membrane preparations prepared from whole mouse uteri and, if possible, epithelial cells, for specific saturable binding of ^{125}I-EGF. Whole segments of uteri from normal, estrogen-treated-ovariectomized, and ovariectomized mice will be examined for autoradiographic localization of EGF receptors. Should cells with specific receptors for EGF be clearly demonstrable, we propose to introduce in situ various concentrations of EGF into the uterine cavity and examine the epithelium at various times after administration. The epithelium will be compared with that of the contralateral uterine horn treated with PBS-0.1% BSA. We shall also attempt with immunohistochemical techniques to localize any epithelial or stromal cells containing EGF-like immunoreactivity. Since relatively high concentrations of EGF have been detected in submaxillary glands and Brunner's glands, it is conceivable that EGF might be present in some of the glandular epithelial cells of the uterus and function locally under appropriate stimuli (estrogen ?). Assessment of a putative uterine EGF will include examination of uterine luminal fluid for EGF receptor-competing activity or immunoreactive material.

The EGF-rich male mouse submaxillary gland is sensitive to circulatory levels of estrogens/androgens. The level of EGF in this gland can be substantially reduced by treatment with estrogen, or further increased by treatment with testosterone. Although control mechanisms may differ for the storage/secretion of EGF in various organs, the latter observation may serve as a significant model to demonstrate that steroidal hormones can markedly control EGF levels.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Polypeptide hormones secreted by endocrine organs or cells are known to exert specific physiological effects at very low serum or tissue concentrations by interactions with complementary receptors. There are numerous clinical cases that indicate that interruption or modulation of any of the sequential events needed for peptides to exert their regulatory role triggers metabolic disorders. Understanding the identity, regulation of, biological response, and mechanism of action of polypeptide hormones is important in proposing logical experimental approaches to assess the interaction of environmental chemicals with biological systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70080-10 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Insu P. Lee Pharmacologist LRDT NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Environmental Chemistry, NIEHS
Laboratory of Developmental Pharmacology, NICHD

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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: (1) The mechanisms by which DNA-damaging agents increase the induction of aryl hydrocarbon hydroxylase activity by TCDD in the prostate glands; (2) Effects of DNA damaging agents on the rates of TCDD cytosolic binding, and nuclear translocation of cytosolic-TCDD receptor in rat prostate glands. (3) Protein analysis of epithelial cells from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors; (4) 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; and (5) susceptibility of testicular tissues to early postnatal treatment with antineoplastic agents.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other 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
	R. A. Ettlin	Guest Worker	LRDT	NIEHS
	M. Matter	Guest Worker	LRDT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) 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). 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 absorbed 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 quantified. Various methylated purine and pyrimidine analog standards were obtained and qualitative and quantitative analyses were developed using MS-GC technique.

(2) TCDD-cytosolic receptor binding and nuclear translocation of TCDD-cytosol receptor. Male Sprague-Dawley rats were treated with procarbazine (MIH) and 12, 18, 24 and 36 hours later, prostate glands were removed, minced, and homogenized in HEDG buffer (2 ml/g tissue) with a polytron at 4°C. The homogenate was centrifuged at 10,000 g for 20 min, and the resulting supernatant was centrifuged at 105,000 g for 1 hour. Sucrose density gradient analysis was prepared by incubating 1 ml of cytosol with 20 μl of [^{3}H] TCDD (in p-dioxane) for 1 hour at 4°C. After incubation, the unbound and loosely bound [^{3}H] TCDD were removed by adding the cytosol to a sextran-charcoal pellet (10 mg of charcoal/mg of dextran, pelleted from HEDG buffer). Dextran-charcoal was resuspended, incubated for 15 min at 4°C, and centrifuged at 4,000 g for 15 min. Aliquots of cytosol were taken before and after dextran-charcoal treatment for determination of total and bound radioactivity. 300 μl of charcoal-treated cytosol were layered on to a linear (5-20%) sucrose gradient, centrifuged at 235,000 g (SW 60 Ti), fractioned, and the radioactivity determined. Internal sedimentation marker (4.6 s) ^{14}C-formaldehyde-induced BSA was added and sedimentation coefficients were then calculated.

In vivo nuclear translocation of [^{3}H] TCDD-cytosol receptor. 1 μCi [^{3}H] TCDD/g body weight was injected i.p. and prostate glands removed at 2, 4, 8 and 16 MRS after injection. The organs were homogenized with an equal volume to the weight of the tissue (0.33 M sucrose in HEDG buffer) and centrifuged at 1600 g for 15 min. Supernatant was saved for cytosol preparation and the nuclear pellet was resuspended and washed 3 times with HEDG buffer. Subsequently, the washed pellet was homogenized in HEDG buffer containing 0.5 M KCl, pH 7.55. The homogenate was allowed to stand for 1 hour on ice to extract nuclear receptors. The extract was then centrifuged at 105,000 g for 1 hour. This supernatant fraction was treated with dextran-charcoal before layering onto 5-20% sucrose density gradients. Gradients were centrifuged and fractionated as sucrose density gradient analysis of the cytosol.

(3) Protein analysis of epithelial cells and spermatozoa from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors following α-chlorohydrin treatment. Adult male F344 rats were treated i.p. once with 100 mg/kg α-chlorohydrin (ACH) and groups of rats sacrificed at 3, 7, 11 and 17 days following treatment. Epithelial cells and sperm from precaput, caput, 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 microscissor. 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 were flushed out by gentle pasteur pipetting. Tubular fragments were then flushed out with a syringe by repeated pumping action and rinsing until no visible sperm were present. 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.

(4) 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 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. Studies of vincristine, 0.15 and 0.6 μg/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 m thickness. Sperm were quantified by counting sperm heads in the testis and epididymides. Fertility was assessed by serial mating.

(5) 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 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, vincristine-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, ABP measurements, time of puberty, and male reproductive capacity.

MAJOR FINDINGS AND PROPOSED COURSE: (1) 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 fold. 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 pHhP-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. Furthermore, whether a significant level of enzyme induction (e.g. AHH) is due to methylation of DNA, mRNA and/or tRNA needs to be studied. Modification of DNA, mRNA and/or tRNA may alter both pretranscriptional and post-transcriptional activity and consequently altered cellular metabolism. DNA, mRNA and tRNA modification on a molecular level is not yet understood. Qualitative and quantitative analysis of methylated nucleotide analogs are being developed for GC-MS to be applied for methylated DNA, mRNA, and tRNA.

(2) TCDD-cytosolic receptor binding and nuclear translocation of TCDD-cytosol receptor. The rates or total [^{3}H]-TCDD binding to cytosolic receptor and those of nuclear translocation between control and treatment group were not statistically different. Therefore, it appears that a significant increase of P_1-450 mRNA transcription was not medicated by an increased rate nor by total number of nuclear translocation of TCDD-cytosolic receptors.

(3) Protein analysis of epithelial cells and spermatozoa from precaput, caput, corpus, and cauda epididymides to identify sperm maturation factors following α-chlorohydrin treatment. The major cytosolic proteins in precaput and caput of

controls were PI 4.8/80k, PI 4.0/50k and PI 4.2/38k. In cauda, however, PI 4.8/80k and PI 4.0/50k proteins were absent, while a PI 5.5/35k protein was present in greater concentration than in the precaput segments. ACH treatment significantly diminished the 80 and 50k proteins in the precaput between days 3 and 7. However, these proteins reappeared by day 17. Protein pattern changes in the caput segment were less dramatic than that observed for the precaput. Thus, SPZ dysfunction after ACH exposure may be due to the altered epididymal tubular cell function which affects SPZ maturation. In addition, a single intraperitoneal injection of α-chlorohydrin to Fischer 344 adult male rats caused desquamation of apical cells of precaput epididymis with subsequent blockage of the lumen. This is evidenced by histopathology and sperm counts in precaput, caput, and cauda epididymis. Sixty to 90% of sperm heads were detached and spermatozoa in the caput and cauda epididymis were significantly depleted by days 7 and 17 respectively.

(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. Vincristine 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 with time. Reduction of epididymal weight was less pronounced. Following vincristine treatment, sequential analysis of testicular tissue allowed one to follow the development of cellular damage, to recognize early changes and characterize the regeneration of cells. The number of tubules damaged correlated well with testicular weight and with the amount of immature germ cells in the epididymal lumina. Sperm head counts correlated well with histologic analysis and confirmed that the cytotoxic action was strongly associated with early and mid-spermatid stages. Fertility was low 1 and 2 weeks after vincristine treatment, indicating an effect of vincristine on SPZ (tail malformation). Sperm head counts did not correlate well with fertility because of the abundance of sperm produced by rats.

(5) 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: Age-related morphologic changes in the testis were associated with exposure to D, P, V and C. CA caused no significant alterations in any age group. Germinal epithelial cell damage and/or loss was severe in treatment age 6d, 16d; P 16d, 45d; moderate in D 24d; P 24d; V 24d, 45d; and mild in P 6d; V 6d, 1d; C 6d. Damage was not apparent in Leydig cells but hyperplasia and hypertrophy occurred in D 6d, 16d; P 6d, 16d. Sertoli cells were altered morphologically in D 6, 16, 24; P 16, 24, 45; V 16, 24, 45. The change associated with V appeared to be a primary effect of the chemical and with D and P could be either a primary effect or secondary cellular atrophy associated with germinal epithelial loss. Significant recovery by end of 12 weeks of serial mating occurred D 24; P 6, 24; V 6, 16, 24; C 6. Delay in onset of reproductive capacity was observed in the

animal groups treated with C 6d, 16d; D 6d, 16d; P 16d, 24d; and V 6d, 16d, 24d, 45d. Decrease in fertility compared after puberty was reached, could be observed in the groups treated with C 6d; P 45d; D 6d, 16d; and V 6d, 45d. In the groups CA 16d, 24d, 45d; C 16d, 45d; D 16d, 45d; P 16d, 24d and V 6d there was slight incidence of increased reabsorptions over the 12 week mating period. D 6d, 16d; P 16d, 45d reduced sperm counts; CA 6d; C 6d, 16d; D 6d, 24d; and P 16d, 24d changed the ABP levels in epididymal cytosol measured in the 12th week of serial mating. Reduced epididymal weight is found with CA 6d, 45d; C 6d, 16d, 24d; D 6d, 24d, 45d; and V 16d, 45d.

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 epididymal sperm maturation processes is important with respect to reproductive biology and also provides clearer insight into how exogenous chemicals may exert their effects on epididymal sperm maturation. Chemicals affecting this sperm maturation process might also suggest new approaches to fertility control.

Modern histological techniques (semi-thin sectioning) 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 histogical 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 deficient in one of these cell types could be produced. Such animals would be valuable models, especially to further explore the physiological role of the Sertoli cells.

PUBLICATIONS

Bronzetti, G., Aeiger, E., Lee, I.P., Suzuki, K., and Malling H.V.: Genetic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in yeast in vitro and in vivo. In Hutzinger, O. (Ed.): Chlorinated Dioxins and Related Compounds. Oxford and New York, Pergamon Press, 1982, pp. 429-436.

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Russell, L.D., Lee, I.P., Ettlin, R., and Malone, J.P.: Morphological pattern of response after administration of procarbazine: alteration of specific cell association during the cycle of the seminiferous epithelium of the rat. Tissue and Cell (In press).

Russell, L.D., Lee, I.P., Ettlin, R., and Peterson, R.N.: Development of the acrosome and alignment, elongation and entrenchment of spermatids as seen in procarbazine treated rats. Tissue and Cell (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70085-06 LRDT

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of In Vitro Models for Assessing Reproductive Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Insu P. Lee Pharmacologist LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Toxicology

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Because both laboratory animals and the commonly used parameters for semen analysis are unreliable predictors of latered human male reproductive capacity, various test approaches utilizing sperm are being developed and validated. Attention is currently directed to the analysis of heterogenously distributed sperm surface proteins appearing during sperm maturation with monoclonal antibodies.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

PI:	I. P. Lee	Pharmacologist	LRDT	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LRDT	NIEHS
	M. Matsuda	Visiting Fellow	LRDT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: 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 sperm (SPZ)-surface proteins at specific stages of maturation, SPZ were obtained from the testis, 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 SOZ in Freund's complete adjuvant. Male mice were subsequently immunized with 1×10^7 SPZ administered i.p. once a week for 4 to 5 weeks followed by a single i.v. injection of 1×10^7 SPZ 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 (SPZ/Ag 14) and centrifuged at 500g for 7 min. Polyethylene glycol was assed 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 37^0C (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 were cloned further.

MAJOR FINDINGS AND PROPOSED COURSE: The analysis of sperm surface proteins 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 the testis, 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 quantified to define sperm maturation factors, and also determine whether any of sperm surface proteins are derived from a specific epididymal epithelium of a specific site of the epididymal tract. Furthermore, a monoclonal antibody will be tested against 2-D gel protein chromatogram obtained from epithelial cells from the precaput, caput, and cauda epididymides to identify a specific protein and/or proteins required for in vitro or in vivo fertilization. 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: Determination of sperm maturation processes is very important with respect to the basic

understanding of the mechanisms of fertilization. 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*.

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 and the Glassware/Media Kitchen perform specific service tasks for the Institute. In addition, Animal Husbandry investigates new methods of containment and disease prevention. Collaborative studies on the use of ventilated cage racks have demonstrated that housing rodents in these systems does not result in elevated metabolism baselines, and probably lowers the baselines. Thus, they may not add an adventitious burden to these parameters in experimental animals. This is an important consideration when new husbandry systems are adopted. Additional collaborative studies with Health and Safety will test the limits of the containment capability of the equipment. These experiments will employ rodent feeding studies that use a highly detectable tracer (fluorescein).

Research efforts of CMB are aimed at disease problems of laboratory animals, principally those that affect dense populations of small rodents. Studies on the natural history of mouse hepatitis virus (MHV) have shown that this infection is widely disseminated and capable of influencing experimental results. MHV is caused by a relatively resistant coronavirus with a rather broad tissue tropism. Important aspects of the natural history of this virus are unknown or poorly understood, including: the duration of active infection, presence or absence of inapparent shedders, duration of patency, latent infection, etc. Studies in our laboratory have shown that experimental infection is easily conferred via the oral, gastric, and intraperitoneal routes. Infection is easily transmitted to naive contacts by actively infected mice, and can also be transmitted by direct exposure of naive mice to the feces of actively infected mice. Immunosuppression of uninfected contacts increases the sensitivity of the assay. Sequential exposure of naive contacts to infected mice to determine the biological length of time that virus is shed is now in progress. Results of these studies have also shown that any increase in the optical density (OD) above a baseline of 0.00 in ELISA serology is significant in the presence of an MHV outbreak or in experimental MHV infection. OD continues to climb until readings approach 2.00. This observation challenges the accepted OD value of 0.17 as the dividing line between positive (more than 0.17) and negative (less than 0.17) ELISA serology. Future studies on MHV will concentrate on virus shedding from infected mice and the use of FA diagnostic techniques.

CMB is also conducting studies on two strains of a naturally occurring rabbit coronavirus (RaCV, PED). These strains appear to be antigenically related and share the same tissue tropisms with the heart being the principal target organ. Preliminary studies on isolated perfused infected hearts show long term damage with incomplete recovery. More studies are necessary to strengthen these

observations. Other target organs include: the eye, and possibly the diaphragm. The importance of this virus to laboratory animal medicine remains uncertain even though small doses of virus (10^7 dilution of infected serum) are necessary to confer experimental infection. Little is known of the natural distribution of the virus. Some analogies may exist between RaCV/PED and human coxsackie and Herpes zoster, especially with respect to myocardial tropism.

General animal disease surveys conducted by CMB on Institute animal colonies and supplier colonies have tracked the prevalence and incidence of rodent diseases. Data from these studies have been used to make decisions on the selection of suppliers, implementation of disease control programs, assisting investigators in the conduct of experiments, and facilitating the acquisition of strains and stocks for experimental use. Two important CMB goals include the enhanced ability to acquire and work with exotic rodent strains and stocks, and the elimination of epidemic disease agents from research colonies. The former will be addressed through the use of a remote isolation unit now in service.

An additional effort has been aimed at determining the level of chemical contamination of animal feed and drinking water. Special interest has been directed at total organic carbon, trihalomethanes (THM), total organic halides, estrogenic substances and heavy metals. Seasonal variation of some contaminants in drinking water, such as THM, has been demonstrated by others. Our studies have shown that some variation in levels of THM does occur, but the significance is poorly understood. The levels of all test chemicals remain low (within EPA acceptable levels). Variation in levels is significantly reduced by distillation. Nutrient analysis has resulted in rejection of one batch of feed because of low Vitamin A levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22100-02 CMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microbial, Nutritional and Chemical Analyses of NIEHS Rodent Diets

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
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:
0.8	.4	.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Six pre and 6 post distilled water samples (4 from RGR and 2 from Bldg. 15) were analyzed for microbial and chemical contaminants. The total trihalomethanes (THMs) level of 117 µg/l (average of 4 samples) in tap water from RGR exceeded EPA's maximum permissible level of 100 µg/l for potable drinking water. The levels of all other microbial and chemical contaminants were within EPA's maximum permissible levels for potable drinking water. Distillation reduced the total THMs including chloroform, a potential carcinogen, from 117 µg/l to an average level of less than 4 µg/liter. In addition, the total organic halides and the elements: chlorine, bromine, sodium, barium, iodine, manganese, magnesium, copper, cerium, calcium, zinc and aluminum were reduced. These results indicate that distillation would provide a more standardized supply of water throughout the year. Levels were higher in the summer (122 µg/l) and fall (211 µg/l) than in the two spring samples (64 and 72 µg/l).

Rodent diets were examined for nutritional content, microbial (Salmonella sp.) and chemical contaminants. Results from each batch of diet were received prior to consumption. All batches that were shipped were accepted; however, one batch was withdrawn by the supplier because of low levels of Vitamin A. Test results were within the acceptable range for the chemical contaminants: arsenic, cadmium, lead, mercury, selenium, chlorinated hydrocarbons, PCB's, BHC's, organophosphates, aflatoxin, and nitrosamines. Each batch was negative for estrogenic activity by the mouse bioassay test and was culture negative for Salmonella sp.

PROJECT DESCRIPTION

METHODS EMPLOYED:

A. Water

Six pre- and 6 post-distilled water samples (4 from RGR and 2 from Bldg. 15) were analyzed to determine the effectiveness of distillation in removing chemical contaminants. Water samples were analyzed for chemical contaminants by contractual agreements with: 1) N. C. State, Nuclear Eng. Dept. (neutron activation analysis for trace elements. 2) Grainger Laboratories, Inc. (methods as specified by EPA for organic and inorganic contaminants in drinking water). Microbial analysis of the water was performed by DRL/CMB/NIEHS.

B. Feed

Each shipment of NIH-31 feed for NIEHS rodents was milled at least 2-3 weeks in advance of use to allow adequate time to determine the microbial, nutritional and chemical quality of the diet prior to consumption. Microbial analyses of the diets were performed by DRL. Diets were analyzed for chemical contaminants by contractual agreements with:

1) Lancaster Laboratories, Inc., Lancaster, Pa. (nutrients, heavy metals and pesticide screens; methods as specified in the Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC, 13th edition).

2) Thermo Electron Corp., Waltham, Mass. (nitrosamines - NDMA, NDEA, NDPA, NDBA, NPIP, NPYR and NMOR; methods - gas chromatography - thermal energy analyzer, GC-TEA).

3) Nutrition International, Inc., East Brunswick, NJ (estrogenic activity; mouse bioassay).

MAJOR FINDINGS AND PROPOSED COURSE: 1) Tap and distilled water were negative for coliforms and Pseudomonas aeruginosa. 2) Chemical contaminants that were reduced by distillation are listed in Table 1. Chemical contaminants detected in tap and distilled water were within EPA's maximum permissible levels for potable drinking water with one exception: the average total THMs level of 117.0 µg/l in the tap water samples from RGR exceeded the permissible level of 100 ppb. Distillation reduced THMs levels to less than 4 µg/l. It was concluded that when the stills are properly maintained, distillation reduces the THMs, including chloroform, a potential carcinogen. In addition, the total organic halides and the elements chlorine, bromine, sodium, barium, iodine, manganese, magnesium, copper, cerium, calcium, zinc, and aluminum were reduced. Distillation would provide a more standardized supply of water throughout the year. THMs levels were higher in the summer (122 µg/l) and fall (211 µg/l) than in the two spring samples (64 and 72 µg/l). Further analyses will be performed to determine the effects of hyperchlorination (15 ppm) on the THMs levels in tap water.

Nutritional and chemical results from each batch of feed were received prior to consumption. All batches shipped were accepted; however, one batch milled for NIEHS was withdrawn by the supplier because of low levels of Vitamin A. The results from the mouse bioassay test for estrogenic contaminants in rodent diets are being analyzed statistically.

We will continue our quality control program for rodent diets; however, we anticipate that this program will be modified on the basis of results and experience.

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. It is essential that experimental animal diets be free of microbial and chemical contaminants and that the diets meet specified nutrient levels.

Table 1. Summary of Comparative Chemical Analysis of Tap and Distilled Water from RGR and Building 15. Mean Values of 4 Samples From RGR and 2 Samples from Building 15.

Substance Assayed	RGR		Bldg. 15	
	Pre	Post	Pre	Post
	Concentration µg/l (ppb)			
1. Total Trihalomethanes	117 (a)	4 (b)	89	6
2. Chloroform	109	3 (c)	77	6
3. Total organic halides	559	80	239	63
4. Elements:				
a) Chlorine	3,248.7	46.6	2,939.2	88.5
b) Bromine	11.9	0.4	11.1	0.8
c) Sodium	10,211.6	108.8	10,276.2	265.5
d) Barium	25.2	1.0*	20.1	1.0*
e) Iodine	3.0	0.5*	2.8	0.5*
f) Manganese	24.5	0.1	8.2	0.6
g) Magnesium	582.2	100.0*	484.7	100.0*
h) Copper	12.5	4.1*	34.8	3.0*
i) Cerium	15.2	0.5*	11.9	0.1*
j) Calcium	21,580.0	200.0*	1,794.0	200.0*
k) Zinc	144.6	4.7	129.9	5.5
l) Aluminum	237.0	6.5	134.9	15.8

* = Less Than

(a) Exceeds EPA's maximum permissible level of 100 µg/l (ppb) for potable drinking water.

(b) Still not operating properly; Pre = 211 and Post = 216. Post results omitted.

(c) Still not operating properly; Pre = 207 and Post = 205. Post results omitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22101-02 CMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Occurrence of Adventitious Infectious Viruses in Research Colonies

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. D. Small Veterinarian 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:
1.5	.5	1.0

CHECK APPROPRIATE BOX(ES)
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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vendor supplied animals, animals received from collaborating investigators, animals raised at NIEHS, and animals under experiment in the Institute were examined for antibodies to several murine viruses.

1. F344 rats supplied by Harlan Industries were regularly positive for antibody to Sendai, PVM and RCV/SDA. Rats frequently had antibody to Sendai and PVM but not RCV/SDA on arrival; however, seroconversion to RCV/SDA and occasionally Sendai occurred in some rats held in isolation for 21 days.

2. Mice supplied by Harlan Industries and Research Triangle Institute had antibody to MHV.

3. Rats supplied by CRBL-Portage had antibody to KRV.

4. Mice received from Texas A&M were free of antibody to 11 murine viruses.

5. Colonies of mice and rats maintained at the CMB-RGR remained free of murine viruses (and other diseases).

6. Rats and mice in 16 rooms in Building 15 and 2 rooms in Building 2 were examined in June 1982 and February 1983 for antibody to murine viruses. Of 15 rooms examined in June 1982 for Sendai virus, 6 were positive (4/8 mice, 2/7 rat). Antibody to Sendai was not found in any animals in all 18 rooms in January 1983. Antibody to MHV was found in 5 of 8 rooms in both surveys. However, the same rooms were not positive on both surveys. Four of seven rooms were positive for RCV/SDA during the initial survey and 10 of 10 were positive during the subsequent survey. Mice (4/8 rooms) and rats (4/7 rooms) were initially positive for PVM. Subsequently 3/7 mouse rooms and 6/10 rat rooms were positive for PVM.

J. E. Thigpen, Microbiologist; C. B. Richter, Vet Med Ofcr, CMB, NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Co-habitation studies, drug induced immunosuppression, sentinel animals and serological methods (HAI, IF, CF and ELISA) are used to determine the incidence (incoming animals) and prevalence (resident animals) of the murine viruses in NIEHS animals.

MAJOR FINDINGS AND PROPOSED COURSE: Supplier surveillance data indicated that: a) incoming rats from Harlan Industries were positive for Sendai, PVM, and RCV/SDA, b) incoming rats from Charles River Breeding Laboratories (Portage) were positive for KRV, c) incoming mice from Harlan Industries and Research Triangle Institute were positive for MHV, and d) mice received from an investigator at Texas A&M University showed no evidence of antibody to 11 murine viruses; however they did have Syphacia obvelata (pinworm).

Health surveillance data collected from two surveys conducted to determine the virus profile of animals housed in NIEHS animal facilities (Bldg. 15 and RGR) revealed the following:

A) In the initial survey (June 1982), 5 of 8 mouse rooms were positive for PVM and MHV, while 4 of 8 rooms were positive for Sendai. Four of 7 rat rooms were positive for PVM and RCV/SDA, while 2 of 7 rooms were positive for Sendai. No viruses were detected at RGR. The oldest resident animals (3-8 months old) in each room were used in the survey.

B) In the second survey conducted in February 1983, 5 of 7 mouse rooms were positive for MHV and 3 of 7 mouse rooms were positive for PVM. Ten of 10 rat rooms were positive for RCV/SDA and 6/10 rooms were positive for PVM. All rooms were negative for Sendai and no viruses were detected at RGR. Sentinel animals housed in the rooms for 4 months were used in this survey.

These data suggest that active Sendai infection is no longer present in the NIEHS colonies; however, MHV and RCV/SDA are present. Further inferences suggested by the data include:

1) Filter caps appear to be highly effective in eliminating Sendai virus from rodent colonies, but less effective in eliminating PVM and coronaviruses.

2) Coronaviruses and PVM have different natural histories from Sendai virus.

3) It may not be possible to eliminate PVM and coronaviruses if animals are continually procured from infected sources.

Major emphasis of CMB's research program is on the natural history and epidemiology of murine coronaviruses.

As a result of these studies, routine acquisition of rats and mice from Harlan Industries, and rats from Charles River (Portage) has been discontinued. Screening of supplier and resident animals will continue as a basis for the development of control and elimination procedures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The quality of research at NIEHS is, in part, dependent upon the use of disease free animals. Viral infections in laboratory animals can profoundly alter biological responses by altering the animals' metabolism, immune response and general health status. CMB's goal is to eliminate spontaneous diseases as sources of variability in research animals.

VIRUS SURVEY, BUILDINGS 2 & 15

Room	Species	MHV		Sendai		PVM		RCV/SDA	
		6/82	2/83	6/82	2/83	6/82	2/83	6/82	2/83
1504	Mouse*	+	+	+	-	-	-	NA**	NA
1509	Mouse	-	+	-	-	-	-	NA	NA
1510	Mouse	-	NT***	-	NT	-	NT	NA	NA
1512	Mouse	+	+	+	-	-	-	NA	NA
1513	Mouse	-	-	-	-	+	+	NA	NA
1516	Mouse	+	+	+	-	+	+	NA	NA
1518	Mouse	+	+	+	-	+	+	NA	NA
206	Mouse	+	-	-	-	+	-	NA	NA
1503	Rat****	NA	NA	+	-	+	-	+	+
1506	Rat	NA	NA	NT	-	NT	+	NT	+
1508	Rat	NA	NA	-	-	+	-	-	+
1511	Rat	NA	NA	-	-	+	+	+	+
1514	Rat	NA	NA	+	-	+	+	+	+
1515	Rat	NA	NA	-	-	-	+	-	+
1517	Rat	NA	NA	NT	-	NT	+	NT	+
1519	Rat	NA	NA	-	-	-	-	-	+
1520	Rat	NA	NA	-	-	-	+	+	+
210	Rat	NA	NA	NT	-	NT	-	NT	+

* Also tested for Polyoma, MVM, Reo-3, GD-VII and MAd; no antibodies detected.

** Not Applicable.

*** Not Tested.

**** Negative tests include: KRV and H-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22102-02 CMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of a Coronavirus from Rabbits

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. D. Small Veterinary Medical Officer CMB NIEHS

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 ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The first coronavirus reported from the rabbit (RbCV) and the first coronavirus associated with cardiomyopathy in any species has been previously described by the PI. Pleural effusion disease (PED) described by Danish workers is thought to be caused by the same virus. From serum containing virus supplied by the Danish workers a pool of serum containing 100,000 rabbit infectious units (RIU) of PED virus per ml and antiserum to PED virus has been prepared. In an in vivo neutralization test PED antiserum neutralized both PED virus (100,000 RIU) and RbCV virus (10 million RIU); however, in a single trial RbCV antiserum neutralized RbCV virus (10 million RIU) but not PED virus (100,000 RIU). Additional trials using RbCV antiserum (from the same lot and a different lot of antiserum) and PED virus will be done. The results thus far indicate similarity between the 2 viruses. The one way neutralization most likely represents differences in potency of antiserum rather than major differences between the virus. The clinical course of the disease and the gross and microscopic lesions appear similar in the 2 groups of rabbits. In a separate study neither virus produced observable effects in nude mice or CD-1 mice and no antibodies were demonstrated against mouse hepatitis virus (a coronavirus) using the ELISA test.

Hearts from acutely infected and recovered rabbits have been examined in vitro to assess physiologic function. Preliminary results suggest long term cardiac dysfunction following infection. This work is continuing and a time course experiment is planned in which hearts will be examined throughout the acute phase of the infection (through 12 days) and during the recovery period (more than 12 days). In conjunction with this work, infected rabbits will be studied for changes in their electrocardiograms and clinical chemical indices, usually related to changes in myocardial tissues, will be measured.

PROJECT DESCRIPTION

OBJECTIVES: 1) Determine the relatedness of rabbit coronavirus (RbCV) and the agent responsible for pleural effusion disease (PED). 2) Determine the effects of RbCV on the rabbit heart.

METHODS EMPLOYED: Serum neutralization studies using rabbits have been done utilizing the Rabbit Coronavirus (RbCV) of Small and the Pleural Effusion Disease (PED) agent from Fennestad (State Serum Institute, Copenhagen). Evidence of infection has been measured by clinical signs and elevation of the rectal temperature beyond 40C. Gross post mortem lesions, confined to the heart, thoracic cavity, peripheral lymph nodes, and sometimes the eyes were compared. Likewise, microscopic lesions were compared.

Isolated (in vitro) heart preparations from virus infected and control rabbits have been studied for the strength of myocardial contraction. The methods of Toy, P.A., et al (Toxicol. Appl. Pharm. 38: 7-17, 1976) were used to study the isolated heart.

MAJOR FINDINGS: From serum containing virus supplied by the Danish workers a pool of serum containing 100,000 rabbit infectious units (RIU) of PED virus per ml and antiserum to PED virus has been prepared. In an in vivo neutralization test PED antiserum neutralized both PED virus (100,000 RIU) and RbCV virus (10 million RIU): however, in a single trial RbCV antiserum neutralized RbCV virus (10 million RIU) but not PED virus (100,000 RIU). The results thus far indicate similarity between the 2 viruses. The one way neutralization most likely represents differences in potency of antiserum rather than major differences between the virus. The clinical course of the disease and the gross and microscopic lesions appear similar in the 2 groups of rabbits. In a separate study neither virus produced observable effects in nude mice or CD-1 mice and no antibodies were demonstrated with mouse hepatitus virus (a coronavirus) using the ELISA test.

Hearts from acutely infected and recovered rabbits have been examined in vitro to assess physiologic function. Preliminary results suggest long term cardiac dysfunction following infection. This work is continuing and a time course experiment is planned in which hearts will be examined throughout the acute phase of the infection (through 12 days) and during the recovery period (more than 12 days). In conjunction with this work, infected rabbits will be studied for changes in their electrocardiograms and clinical chemical indices, usually related to changes in myocardial tissues, will be measured.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1) The work will aid in clarifying the relatedness between RbCV and PED. 2) This is the first coronavirus reported in the rabbit and the first coronavirus associated with myocardial lesions. While the coronaviruses are considered to be species specific, they vary in their tissue tropisms and they share many similar properties. This infectious process allows for the study of myocardial activity in the presence of an infection which apparently has the myocardium as its major, if not, sole target. This work suggests a similar study with another virus, Virus III of Rivers, a Herpes virus which also has a tropism for the heart and is probably identical to Herpes cuniculi. Virus III per se is no longer available, but H. cuniculi is.

Further, if the isolated heart preparation proves to be successful, it opens the way for the study of drugs and toxic substances on the diseased heart. The damage to the rabbit heart by RbCV has a corollary in the human heart with the Coxsackie viruses, Mycoplasma pneumoniae, influenza virus, Herpes zoster, and possibly other infectious agents.

PROPOSED COURSE: Continuation with emphasis on the effects of the viruses on the myocardium and the comparison of these effects with those of recognized human disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22103-01 CMB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biology of Murine Viruses (MHV)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
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:
0.4	.2	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse hepatitis (MHV) has been reproduced experimentally in nu/nu mice by experimental infection (mortality 5-7 days) and by cohabitation with experimentally MHV infected CD-1 mice (mortality 40-50 days). Disease free CD-1 mice seroconverted to MHV when cohabited with naturally infected B6C3F1 mice. MHV is shed in the feces of naturally infected B6C3F1 mice and the disease can be transmitted to MHV free CD-1 mice by direct fecal exposure. CD-1 and nude mice can be experimentally infected by the intra-nasal (i.n.), intraperitoneal (i.p.), oral or gavage (stomach) routes. ELISA optical density (O.D.) values in CD-1 mice at 19 days post MHV-infection by these routes were: i.n. (0.27, 0.10, and 0.12), i.p. (1.47 and 1.00), oral (1.21, 0.89, and 1.50), and gavage (0.47 and 0.00). ELISA O.D. values 0.17 and higher are considered positive in serological screening results. ELISA O.D. values were more uniform and higher in mice infected by the oral and i.p. routes. Intra-nasal and gavage exposure produced variable results.

PROJECT DESCRIPTION

METHODS EMPLOYED: Co-habitation studies, immunosuppression with cortisone acetate (200 mg/kg of body wt), experimental infection, and serological methods (HAI, IFA, CF and ELISA) have been or will be used to study the natural history and the epizootiology of MHV.

Co-habitant virus free mice were housed in the same cage with MHV naturally infected mice. At weekly intervals post housing, the co-habitants were removed and isolated in a Horsfall unit or a laminar flow hood to prohibit further potential exposure to MHV, and additional virus free co-habitant mice were added. Feces and bedding from infected mice were also collected and placed in a sanitized cage containing virus free mice. At 24-28 days post exposure the cohabitants were bled for viral serology. Nu/nu mice were experimentally infected with MHV or were co-habited with experimentally infected CD-1 mice and observed for 60 days for clinical signs of MHV.

MAJOR FINDINGS AND PROPOSED COURSE: These studies are in progress. Preliminary findings are: 1) MHV has been successfully reproduced in the nu/nu mouse by experimental infection (mortality 5-7 days) and by co-habitation (mortality 40-50 days) with MHV infected CD-1 mice. 2) CD-1 mice cohabited with MHV naturally infected B6C3F1 mice readily seroconvert to MHV positive. 3) MHV is shed in the feces of B6C3F1 infected mice and the disease can be transmitted to virus free CD-1 mice by direct fecal and bedding exposure. 4) CD-1 mice can be experimentally infected by the intra-nasal, oral, gavage (stomach), or i.p. routes. ELISA optical density (O.D.) values in CD-1 mice at 19 days post MHV-infection by these routes were: Intra-nasal (0.27, 0.10, and 0.02), i.p. (1.47 and 1.00), oral (1.21, 0.89, and 1.50) and gavage (0.47 and 0.0). O.D. values of 0.17 or higher are considered positive when virus serology profiles are interpreted; however, evidence shows that in an epidemic lower values are significant. ELISA O.D. values were more uniform and higher in mice infected by the oral and i.p. routes. Intra-nasal and gavage exposure produced variable results.

We will continue to study and evaluate the factors which affect the distribution of infectious MHV among mice. Specifically, we will attempt to determine whether an inapparent carrier state exists in experimentally infected mice. If so, for how long? Preliminary evidence indicates that normal infected mice stop shedding the virus between 14-21 days post infection.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The quality of research at NIEHS is partly dependent upon the use of pathogen free animals. Viral infections in laboratory animals can profoundly alter biological responses by altering the animal's metabolism, 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 animal facilities by causing mortality in nu/nu mice and by altering immunological studies in clinically normal mice. Little is known about the natural history and epidemiology of MHV. It is important that we study the transmission and the stability of this virus in our environment if we are to eliminate the disease. CMB's ultimate goal is to eliminate spontaneous diseases as sources of variability in our animals.

TOXICOLOGY RESEARCH AND TESTING PROGRAM

TOXICOLOGY RESEARCH AND TESTING PROGRAM

Summary Statement

The Toxicology Research and Testing Program, the National Institute of Environmental Health Sciences component of the National Toxicology Program, develops scientific information about potentially toxic and hazardous chemicals that is 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 and continuing aims center on:

-- Expanding toxicological profiles of the chemicals nominated, selected, and tested.

-- Increasing as necessary and as funds permit, the number and rate of chemicals tested.

-- Developing and validating a series of tests/protocols more appropriate for research/regulatory needs.

-- Using 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 are Cellular and Genetic Toxicology (Dr. R. Tennant), Chemical Pathology (Dr. E.E. McConnell), Systemic Toxicology (Dr. B. Schwetz), and Carcinogenesis and Toxicology Evaluation (Dr. B. Schwetz). The management Branches involve Program Operations (Dr. J.F. Douglas), Program Resources (Dr. W. Jameson), 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 investigation and perhaps 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 studies 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 is being used 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 initiated and integrated certain advancements into the long-term (2-year) carcinogenesis studies.

Biomathematical simulations aimed at improving the basic experimental design of the two-year carcinogenicity studies were conducted. The primary objective was to maintain the power of the current design, while improving the design from the standpoint of goodness of fit and low-dose extrapolation. For a total sample size of 200 the following design was recommended: 50 controls, 30-40 animals at a dose of 20-30% of the estimated maximum tolerated dose (EMTD), 60-70 animals at a ½ EMTD dose, and 50 animals at the EMTD. This design is being implemented where appropriate.

Traditional pathology procedures in two-year rodent carcinogenesis studies require that 42 sections from 32 tissues be examined microscopically from all animals. Analyses of the results from previous NCI and NTP carcinogenesis studies allow TRTP to modify the number of tissues examined without compromising the ability to detect chemically-induced neoplasms. Further, the quality of the toxicologic pathology will be markedly improved through examination of some animals at a time period (12-18 months) earlier than 24 months, when normal aging lesions sometimes 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 Studies. A major objective will be to assess the ability of selected chemicals to function 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.

Particular chemical class studies have been designed to better define structure activity-toxicity 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.

MT. SINAI SCHOOL OF MEDICINE - NEW YORK, NEW YORK
(CONTRACT - NIH - NO1-ES-9-0004)

TITLE: Investigation of the immunobiological and toxicological effects of PBB in Michigan farmers and chemical workers

CONTRACTORS PROJECT DIRECTORS: J. G. Bekesi, Ph.D. and I. Selikoff, M.D.

PROJECT OFFICER (NIEHS): John A. Moore

DATE CONTRACT INITIATED: June 29, 1979

CURRENT FUNDING LEVEL: $282,000

PROJECT DESCRIPTION

OBJECTIVES: The specific objectives of this contract were to examine a larger group of Michigan residents exposed to PBB following the 1973 accident and to: 1) verify the existence of the previously reported toxicological and immunological alterations; 2) to conduct a detail analytical analysis of PBB body burden in exposured individual with emphasis on quantitating the PBB isomers in adipose and serum samples; 3) to correlate immune dysfunction with other reported symptoms or conditions which occur in individuals with the "toxic PBB syndrome"; and 4) to attempt to characterize in-depth immune alterations observed and elicidate the underlying mechanism.

METHODS EMPLOYED: Between 1980-1982 a survey was initiated in which 336 Michigan farm residents, 29 PBB-exposed chemical workers, 156 individuals from the general Michigan population and 80 Wisconsin control farm family members were evaluated for immunological and toxicological findings. In addition, 109 New York City residents were examined immunologically as control individuals.

All Michigan and Wisconsin residents enrolled in the study received a standardized health evaluation with a specific focus on parameters allegedly associated with the toxic PBB syndrome. Specific parameters examined were as follows: immune function assessment; liver enzyme panel; neurological and/or neuropsychiatric evaluation; qualitative and quantitative evaluation of porphyrins in urine; dermatological examination; and quantitation of serum and adipose organohalide body burdens (PBB, PCB, DDT, DDE and etc.). The contractor also quantitatively evaluation the compartmentalization of PBB in specific subsets of leukocytes and serum components.

During the past year, the contractor has focused on an extensive indepth evaluation of individuals demonstrating altered lymphocyte function during the 1979 - 1982 reevaluation with emphasis on individuals demonstrating impaired immunological responsiveness to mitogens and alloantigens, altered cell surface marker distribution, and altered immunoglobulin levels. The purpose of this limited indepth evaluation was to characterize the immune alterations in those most seriously affected and to determine the underlying mechanism. To achieve this objective, the principal investigator has evaluated the responsiveness of

lymphocytes from these individuals to biologic response modifiers; the ability of their cells to express natural killer cell activity, an indepth characterization of T lymphocyte subsets using monoclonal antibodies for cell surface marker characterization; and an in vitro evaluation of antibody synthesis and regulation.

MAJOR FINDINGS AND PROPOSED COURSE: During the past six months the contractor has focused on correcting statistical analysis deficiencies observed during the last review of this contract. The principal investigator and his staff have made considerable progress with the detailed statistical analysis of the immune function abnormalities, in developing criteria for determining which immune function parameters are abnormal using a 95% confidence interval based on control values, and in cluster analysis to determine how immune abnormalities clustered among individuals and families. Some progress was also made in correlating immune dysfunction with clinical abnormalities.

Clinical Findings: Of the 336 adult farm residents and 29 chemical workers evaluated, 134 (37%) gave a history of neurological symptoms which included frequent headaches, dizziness, paresthesias, loss of balance, fatigue, nervousness, and loss of memory. In addition, 149 (42%) expressed musculo-skeletal symptoms including joint pain and swelling of joints. Neurological and musculoskeletal symptoms were persistent from the earlier observation in 1976 and are the two most prevalent symptoms reported. A reevaluation of clinical and neurological symptoms was conducted on 40 of the original 45 individuals studied during the 1976 study and 19 of these individuals (47.5%) continued to present a history of neurological symptoms of the same nature as they had previously reported. Fourteen (35%) of the 40 individuals restudied continued to complain of joint pain often associated with swelling of joints.

Clinical chemistry evaluation of these 40 individuals indicated liver function tests remained constant and normal from 1976 to 1980 with the exception of SGOT. Most of the study subjects both past and present have normal liver function tests and this also applies to both Michigan general population groups.

A large proportion of the 336 Michigan farm residents continued to exhibit cutaneous hyper reactivity to the recall antigens mumps (28-51%) and to varidase (45-62%). It should be noted that some general hypereactivity was noted in the general Michigan population to these two antigens. An evaluation of immunoglobulin levels revealed that 60% of the Michigan dairy farmer residents had abnormal levels of IgG as compared to an "abnormality" rate of 20% in the Michigan chemical workers and 23% in the general Michigan population. Some abnormal expression of IgM and IgA were also seen in a few individuals although the frequency does not approach that seen with IgG. Similar results were found when one examined serum complement (C) components. For example, abnormal values of C_3c were found in 58% of the Michigan farm residents as compared to 26% of tOe chemical workers and 24% of the Michigan general population using the 95 percentile cut-off for Wisconsin farm residents.

Examination of cell-mediate immunity and the cell types involved revealed the following data. Michigan farm residents and chemical workers expressed a significantly decreased level of T lymphocytes and a statistically significant increase in circulating level of null cells (i.e., cells without either T or B

cell surface markers). The mean null cell percentage in Wisconsin dairy farmers was 11.8 as compared to 21.3 and 25.3 in Michigan farm residents and chemical workers, respectively. This alteration in the peripheral expression of T lymphocyte subpopulations may suggest some alteration in maturation or cell surfaces by PBB exposure. In addition, Michigan farm family members and chemical workers also demonstrated significantly depressed proliferative responses of lymphocytes to PHA, PWM, CON A and in mixed leukocyte culture. Using cluster analysis, the immune dysfunctions seen in the Michigan dairy farm population were divided into four subgroups as follows: 1) Individuals with low lymphocyte response to PHA, CON A, PWM with normal percentages of T, B and null cells (n = 52/331); 2) Individuals with low lymphocyte response to PHA, CON A, PWM with abnormal percentages of T and null cells (n = 30/331); 3) Individuals with increased levels of immunoglobulins G, A and C_3 with normal percentages of T, B and null cells and normal lymphopro-liferative responses (n = 26/331); 4) Individuals with increased levels of immunoglobulins G, A and C_3 with abnormal percentages of T, B and null cells and impaired lymphoprolifeTative responses to PHA and CON A (n = 25/133); and 5) Individuals with no or only 1 abnormal immune parameter (n = 199/331). Attempts were then made to correlate clinical changes within each of these subgroups. Individuals with immune abnormalities also more frequently demon-strated the clinical symptoms previously described.

The clinical findings most frequently associated with Subgroup 1 were neurological and musculoskeletal system involvement. Subgroup 2 had a high percentage of neurological and musculo-skeletal symptoms and in addition 4 individuals (13%) developed neoplasias since the 1976 field survey. This observation was interesting since the individuals in this subgroup had both a numerical and functional impairment of T and B lymphocytes. Individuals in Subgroup 3 showed an elevated ASO titer both in frequency and level of response. These individuals also had increased serum levels of IgG and IgA. Subgroup 4 represents 25 dairy farmers with the most serious multiple immune dysfunctions. These are polycolonal hypergammaglobulinemia, increased ASO titers, reduced T cell populations and functions, and increased null cell populations. These abnormal immunological findings were accompanied by a significantly hypersen-sitivity in vivo response to the recall antigens mumps and varidase. Multiple clinical symptoms primarily characterized by abnormalities in neurological and musculo-skeletal systems were predominant in this group. Six farmers (24%) developed cancer since the 1976 survey. The PBB levels in this group ranged between 0.6 to 70 and remained unchanged or increased since the 1976 survey.

A cluster analysis of immunological abnormalities by husband and wife revealed that there was a strong correlation of immunologic abnormalities in both partners suggesting that exposure and not genetic predisposition was probably the underlying factor in this abnormality. Finally, the principal investigator has provided very nice scatter plots of the comparison of immuno-logical measurements among 40 of the 45 individuals studied in 1976 and again in 1981. For all immunological abnormalities examined at both time periods there was a high degree of correlation and significant correlation coefficients were provided. These data indicate that the abnormalities observed in 1976 have persisted through 1981. The degree of correlation between these two datasets in the retested individuals was extremely encouraging and speaks to the generally high quality of the studies performed by the PI and his staff.

Preliminary data from the indepth characterization of 25 subjects from subgroup 1 and 20 subjects from subgroup 4 are preliminary and as follows:

1) Individuals within subgroup 4 have a marked increase in the proportion of lymphocytes positively stained for cytoplasmic IgG and IgM, concomitant with an increase in IgM and IgG secretion in vitro. 2) A strong correlation was found between rheumatoid factor positive responses and joint pain and swelling in this subgroup. Hypersensitivity to the recall antigens mumps and varidase was persistent and accompanied by elevated ASO titers and immunoglobulin levels in subgroups 3 and 4. 4) Natural killer cell activity was markedly lower than the control population in individuals in both subgroup 1 and 4.

Progress in the chemistry area has been good and can be summarized as follows: These investigators have confirmed the initial observation that the -lipoproteins are the major carrier proteins for PBB and determined that approximately 75% of protein-bound PBB is carried by these proteins. Serum albumin and other serum protein constituents serve to carry little PBB. The in vitro mixing of ^{14}C-label hexachlorobiphenyl with human sera provides a good model for investigation the distribution of these components in environmentally contamined subjects. The techniques developed for negative ionization mass spectroscopy has been validated and are currently being used in the final phases of the study. In summary, the overall progress on immunological data analysis, analysis of the serum protein distributions of PBB and preliminary data on the indepth study appears to be excellent.

SIGNIFICANCE TO BIOMEDICAL RESEARCH IN THE PROGRAM OF THE INSTITUTE: The complex relationship between PBB exposure, PBB body burden, PBB distribution among lymphoid cells, clinical symptoms associated with PBB expoure and immune alteration seen in Michigan farmers and chemical workers exposed to PBB still remains complex. Individuals in subgroups 2 and 4 have the most clinical symptoms which may or may not be PBB related, but was of interest since they are found in subgroups with the most serious forms of immune dysfunction. The long-term health consequences, if any, in individuals exposed to PBB is still not fully understood.

FOOD AND DRUG ADMINISTRATION
National Center for Toxicological Research (NCTR)
Bethesda, Maryland 20205
(222Y01-ES-00052)

TITLE: Teratogenicity Testing of Chemicals for the National Toxicology Program

CONTRACTOR'S PROJECT DIRECTOR: Carole A. Kimmel, Ph.D.

PROJECT OFFICER (NIEHS): John A. Moore, D.V.M.

DATE CONTRACT INITIATED: May 1, 1980

CURRENT LEVEL: $655,000

PROJECT DESCRIPTION

OBJECTIVES: The objective is to provide testing of chemical agents for their potential to cause teratogenicity and developmental toxicity. This effort is attributed to reproductive testing of chemicals designated for study by the National Toxicology Program.

METHODS EMPLOYED: Testing of environmental chemicals in at least two of four species of pregnant laboratory animals (mice, rats, rabbits, hamsters) and developing data on the susceptibility of their embryos/fetuses to developmental toxicity following the administration of test chemicals during development.

MAJOR FINDINGS AND PROPOSED COURSE: The testing of chemicals for teratogenicity has continued on contract in FY 1983. Fifteen studies on 10 chemicals have been completed, and an additional 9 studies on 5 chemicals are underway (Table 1). Four abstracts on the teratologic evaluation of diphenhydramine, 5-hydroxytryptophan and diethylhexylphthalate were presented at the Teratology Society meeting in June, 1983. NCTR's current contract ends September, 1983, and a new contract is being negotiated to begin October 1, 1983. Selection of chemicals for conventional teratology testing for FY 1984 is underway and is coordinated with the selection of chemicals for and results of the short-term in vivo reproductive toxicity assay and the sperm morphology/vaginal cytology assay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The goal is to determine the relative safety of those chemicals to which a wide segment of the population is exposed. In this case, the population consists primarily of women of childbearing age, specifically pregnant women.

Table 1
Developmental Toxicity Studies Completed or in Progress in FY 1983[a]

Chemical	CAS No.	Species	Route[b]	Fetotoxicity: Prenatal Death	Fetotoxicity: Reduced Fetal Weight	Increased Malformations	Maternal Toxicity
Conventional Teratology Studies Completed in FY 1983							
Bendectin®	8064-77-5	Rat	Oral[c]				
Carbon disulfide	75-15-0	Rat	Oral[c]				
		Rabbit	Oral[c]				
Chlorpromazine hydrochloride	69-09-0	Mouse	Oral	+(30 mg/kg)	+(15 mg/kg)	+(30 mg/kg)	+(5 mg/kg)
		Rat	Oral	+(30 mg/kg)	+(5 mg/kg)	--	+(15 mg/kg)
Di(2-ethylhexyl) phthalate	117-81-7	Mouse	Diet D/0-17	+(.10%)	+(.15%)	+(.05%)	+(.10%)
		Rat	Diet D/0-17	+(2%)	+(.5%)	--	+(.5%)
Hexamethyl-p-rosaniline	548-62-9	Rabbit	Oral	+[d]	+(.5 mg/kg)	--	+(.5 mg/kg)
5-Hydroxytryptophan	56-69-9	Mouse	Oral	--	+(300 mg/kg)	--	+(150 mg/kg)
		Rat	Oral	+(300 mg/kg)	+(300 mg/kg)	+(300 mg/kg)	+(150 mg/kg)
Isoproterenol	51-30-9	Rat	S.C.	+(1.0 mg/kg)	+(.25 mg/kg)	--	+(.25 mg/kg)
Oxytetracycline hydrochloride	2058-46-0	Rat	Oral	--	+(1200 mg/kg)	--	+(1200 mg/kg)
Phenol	108-95-2	Mouse	Oral	--	+(280 mg/kg)	--	+(280 mg/kg)
		Rat	Oral	--	+(120 mg/kg)	--	--
Sulfamethazine	57-68-1	Rabbit	Oral[c]				
Conventional Teratology Studies - Testing in Progress in FY 1983							
Bisphenol A	80-05-7	Rat	Oral				
		Mouse	Oral				
Ethylene glycol	107-21-1	Rat	Oral				
		Mouse	Oral				
Hydrochlorothiazide	58-93-5	Rat	Oral				
		Mouse	Oral				

[a] A + indicates a positive effect; number in parentheses is the lowest dose at which the effect was detected.
[b] Dosing days 6-15 in rats and mice, days 6-19 in rabbits unless otherwise indicated.
[c] Final report not completed.
[d] Significant trend with no pairwise difference.

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.

Six other contract laboratories, Battelle-Columbus, Microbiological Associates, EG&G Mason, International Research and Development Corporation, Bioassay Systems, and Hazleton Laboratories America, have begun automated data entry this fiscal year.

TDMS support of pathology by NIEHS scientists, quality assurance of pathology, and other selected NIEHS studies are commencing.

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.

CARLTECH ASSOCIATES, INC.
Columbia, MD 21044
N01-ES-2-5020

TITLE: Support for Preparation of Toxicology and Carcinogenesis Studies Technical Reports

CONTRACTOR'S PROJECT DIRECTOR: Marcia Rodgers

PROJECT OFFICER (NIEHS): James E. Huff, Ph.D.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT LEVEL: $527,000

PROJECT DESCRIPTION

OBJECTIVES: To provide technical reports preparation services for approximately 50 reports per year.

METHODS EMPLOYED: Preparation of technical toxicology and carcinogenesis reports on chemicals shall overlap in that 10-20 reports will be in varying stages of completion. Maintenance of a well-organized data file on each chemical for which a technical report is being or will be prepared. Word processing equipment is used for technical report preparation and for transmission of information to and from NTP offices.

MAJOR FINDINGS AND PROPOSED COURSE: Technical Reports preparation support services are provided to Chemical Managers, Discipline Leaders, and the Toxicology Research and Testing Program Technical Reports Review Committee, in writing, editing, and doing successive draft revisions in close collaboration with National Toxicology Program staff. Designated groups of reports are prepared in completed form with strict deadlines for review by the in-house Staff Review and subsequently by the NTP Board of Scientific Counselors Peer Review Panel. Final camera-ready material is provided for printing of Technical Reports by a Government Printing Office contract printer.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: A necessary resource to the National Institute of Environmental Health Sciences, National Toxicology Program for the organization of laboratory collected data and materials, collection and preparation of reports to be drafted and printed for chemicals being tested for the NTP Toxicology and Carcinogenesis Program.

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: $300,000

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 46,321 (May 1983) 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 46,321 documents equals 13,813.

DEPARTMENT OF ENERGY - OAK RIDGE NATIONAL LABORATORY
(222 YO1-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 29,399 (as of May 1983) records, the majority of which are available online from TOXLINE AND RECON. The number of unique chemicals identified from these 29,399 documents equals 5,422.

ETIC, located at NIEHS, has established a microform document library containing copies of 23,999/29,399 (82%) 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.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
Lyon, France
NO1-ES-1-5009

TITLE: Establishment and Maintenance of an International Register of Persons Exposed to Phenoxy Acid Herbicides and Contaminants

CONTRACTOR'S PROJECT DIRECTOR: Rodolfo Saracci, M.D.

PROJECT OFFICER (NIEHS): J.E. Huff, Ph.D.

DATE CONTRACT INITIATED: July 20, 1981

CURRENT LEVEL: $122,705

PROJECT DESCRIPTION

OBJECTIVES: To identify, learn about, and assess the suitability for epidemiologic study of cohorts (outside of the United States) with defined exposure to phenoxy herbicides and contaminants, principally chlorinated dibenzo-p-dioxins. To identify a scientist in each country having at least one potential cohort who is interested in collaborating with IARC on this project and to learn from these contacts about other potential cohorts.

METHODS EMPLOYED: Phenoxy herbicides and chlorophenol manufacturers outside the United States are being identified from published literature and from scientists and company officials. Potential epidemiologic collaborators are identified and the IARC plan is described with intent of enlisting assistance in learning about potential cohorts.

MAJOR FINDINGS AND PROPOSED COURSE: Major factories and many collaborators have been identified. Key contacts have been made and plans discussed for the collection of specific information on the cohorts. The proposed course is to assemble more specific information about the company are available and suitable for an IARC Registry, and perhaps ultimately for an epidemiologic study.

SIGNIFICANCE TO THE PROGRAM OF THE INSTITUTE: The initiation and maintenance of an international registry of persons exposed to phenoxy acid herbicides and contaminants, principally chlorinated dibenzo-p-dioxins. These data will be made compatible with those being collected in the United Stated by the National Institute of Occupational Safety and Health.

TITLE: Information Resources

PROJECT OFFICER: Joan W. Chase

DATE CONTRACT INITIATED: October 1, 1982

CURRENT ANNUAL LEVEL FOR ALL CONTRACTS: $50,000

BIOSPHERIES, INC., ROCKVILLE, MD — NO1-ES-28028
CONTRACTOR'S PROJECT DIRECTOR: Frances P. Lederer

CAPITAL SYSTEMS GROUP, KENSINGTON, MD — NO1-ES-28029
CONTRACTOR'S PROJECT DIRECTOR: William Hassler

ENVIRONMENTAL CONTROL, INC., ROCKVILLE, MD — NO1-ES-28030
CONTRACTOR'S PROJECT DIRECTOR: John R. Strange

ENVIRONMENTAL RESOURCE CENTER, SILVER SPRINGS, MD — NO1-ES-28031
CONTRACTOR'S PROJECT DIRECTOR: Brian Karnovsky

GEOMET TECHNOLOGIES
CONTRACTOR'S PROJECT DIRECTOR: Harriet Stern — NO1-ES-28032

PROJECT DESCRIPTION

OBJECTIVES: To supply technical information services to the staff of TRTP/NIEHS mainly and to the NIOSH and NCTR portions of the NTP on an ad hoc basis.

METHODS EMPLOYED: At present the contract is vested entirely in Environmental Resources Center which does library searches for journal articles, and xeroxes and delivers two copies to the Project Officer for distribution to the Chemical Manager and to Carltech for report writing. Special translating services are also supplied on an ad hoc basis.

MAJOR FINDINGS AND PROPOSED COURSE: Information services are supplied as needed. Other services (i.e. customized searches, reports) can be done as tasks are required.

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. Growth of females was depressed at all dose levels with the high dose group actually weighing less at the end of the exposure regime. The growth rates of males exposed to 5.0 ppm was markedly depressed during the first half of the exposure period, but the males seemed to recover. In spite of marked weight changes in the high dose male group, the only functional change noted was a loss of elastic recoil indicative of a mild obstructive lesion.

Min-U-Sil, a crystalline quartz dust, was used to compare lung function, composition, and morphology in rats developing silica-induced lung disease. Groups of 24 male Fischer-344 rats were exposed to 0, 2, 10, or 20 mg/m^3 and assessed after 3 months, 6 months, or 6 months after a 6 month exposure. Little significant physiologic or radiographic abnormality was seen in any exposure group after 13 or 26 weeks of exposure. However, the presence of birefringent silica particles in alveolar macrophages and lymphoid tissue after 13 weeks was sufficient to correctly identify each group. After 26 weeks, epithelial hyperplasia and granulomata were present. Hydroxyproline was elevated at both time points. Six months later, radiographic and functional changes were also observed along with the continuing biochemical and anatomical changes.

In summary, effects of inhaled silica were seen at all time points. Early effects consisted of changes in connective tissue levels, inflammatory cell proliferation and formation of small granulomata. These effects increased in severity with time and concentration. A restrictive lung lesion was seen with functional measurement only in the most challenged animals a year after the exposures began. With silica, histopathologic and biochemical measurements were more sensitive to the exposure than were functional measurements.

In another study, Fischer-344 rats were exposed at 0, 0.3, 1, or 2 mg Cd/m^3 (cadmium chloride aerosol) for 6 hr/d, 5 d/wk for a scheduled 62 exposure days. All the rats in the high dose chamber died by the 45th exposure day. The 1.0 mg/m^3 airborne concentration was highly toxic. Static lung volumes, compliance, and forced expiratory flow rates were dramatically reduced, indicating a prominent airway and parenchymal disease consistent with massive fibrosis. The 0.3 mg/m^3 rats also exhibited evidence of fibrosis but of a mild interstitial nature.

The final study under this NTP contract investigates the effects of tungsten carbide, cobalt, and a mixture of these two metals in the approximate concentrations found in grinding and cutting tools. Male Fischer-344 rats will be exposed to either 1 mg/m^3 cobalt metal dust, 15 mg/m^3 tungsten carbide dust as tungsten, and the two metals combined at the same respective airborne concentrations. Exposures and biological responses will be performed in 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.

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.

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 142 chemicals is being studied under NTP Basic Ordering Agreement. 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 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 designed to help define toxicity from "PUVA" therapy, a therapy which involve the combination of UVA and psoralen for the treatment of psoriasis and vitiligo (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. Planned work in the area of chemical disposition and metabolism has been completed. Significant progress was made in genetic toxicology testing and chronic in vivo studies on two dyes and two parent amines were initiated. The prechronic testing of a third dye was completed (Dr. Mennear).

- The use of microencapsulation to stabilize volatile or reactive compounds for toxicologic studies in dosed feed preparation is being evaluated in collaboration with Drs. Jameson and Goehl. 2,6-Xylidene and trichloroethylene have been encapsulated. The effect of microencapsulation on the chemical stability and bioavailability of these compounds is being determined (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 the chronic phases (Dr. Chhabra).

- A study to compare the general and immunologic toxicity and carcinogenicity of nickel oxide, nickel sulfate, and nickel subsulfide has been initiated. Nickel has been labeled as a "human carcinogen", but the relative toxicity of the common nickel salts is not known. These studies are designed to relate rodent toxicity and carcinogenicity with human epidemiologic data on nickel (Dr. Dunnick).

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).

- The results of NTP toxicity and carcinogenicity studies with various halogenated aliphatic hydrocarbons are under review for determining structural features essential for their carcinogenicity and/or nephrotoxicity. The biochemical effect of these compounds on the kidney both in vivo and in vitro will be investigated. Since high mortality was encountered with a large number of these compounds during the two-year studies, the results obtained here will be used to define appropriate dose levels for chronic tests with similar compounds (Dr. Abdo).

- Two stage carcinogenesis in the F344 male rat urinary bladder is being studied in collaboration with Dr. Maronpot. A study to determine subcarcinogenic dose levels for N-methyl-N-nitrosourea initiation of bladder tumor in the F344 rat is scheduled to begin during the summer of 1983 (Dr. Melnick).

- Studies are underway to determine whether reactive intermediates of oxygen reduction are generated in rodent livers due to peroxisomal proliferation from treatment with di-2(ethylhexyl)phthalate (Dr. Melnick).

- The effects of neurotoxic pesticides on the in vitro polymerization and enzymatic activities of microtubes is being examined as a model system for the mechanism of action of these agents in vivo. Correlation of in vitro and in vivo mechanisms may lead to development of a simplified method for screening certain neurotoxic agents (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).

- 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).

- There is a high spontaneous incidence of mononuclear cell leukemia (MNCL) in Fischer 344 rats that may cause up to 50% mortality in 18-26 month old rats. In this important test animal, both spontaneous and chemically-induced MNCL are being investigated to determine their impact on interpretation of tumor incidence and tumor and non-tumor pathobiology in chronic toxicity tests, where the hematopoietic system may be a target organ. These studies are being performed in collaboration with Drs. Dieter, Maronpot and Boorman (Dr. French).

- In vitro methods to study the absorption and metabolism of chemicals applied to rodent skin are being evaluated for the purpose of establishing the capabilities to perform these tests in-house (Dr. Eastin).

- Studies using an in situ luminal perfusion technique to measure intestinal absorption rates of 4,4-Thio-Bis-6-t-butyl-m-cresol) in rats were completed in collaboration with Dr. Birnbaum (Dr. Eastin).

- Diagnostic tests for assessing renal function in vivo and in vitro are being developed and validated. Molecular and physiological mechanisms of acute and chronic chemical nephropathies caused by halogenated alkyl compounds are being studied using whole animal and in vitro techniques (Dr. Kluwe).

- Biochemical mechanisms of infertility in males produced by the pesticide 1,2-dibromo-3-chloropropane are being studied. High exposures produce testicular atrophy, while low doses impair sperm energy metabolism, possibly via metabolism to a chlorinated analogue of a normal energy substrate (Dr. Kluwe).

- Male infertility induced by the phthalate esters (e.g., di[2-ethylhexyl]-phthalate, butyl benzyl phthalate) is being characterized. The mechanism appears to involve an alteration in zinc metabolism and, in some instances, direct endocrinological effects of the chemicals (Dr. Kluwe).

- The effects of di(2-ethylhexyl)phthalate and other 2-ethylhexanol-containing chemicals on liver structure, function and biochemistry are being evaluated. Many of these agents are hepatocarcinogens and may exert such effects via an alteration of liver oxygen metabolism. The interrelationships between altered liver biochemistry, hepatotoxic response, and lipid metabolism are being studied (Dr. Kluwe).

Other Activities:

Dr. Kluwe: Continues to monitor and develop the NTP Phthalate Ester Program. Currently a member of the program subcommittee for the IUPHAR-sponsored International Conference on Phthalates (August, 1984). TRTP representative on the Hazardous Waste Information Evaluation Subcommittee (HWIES) of the DHHS CCERP.

Dr. Kluwe: Was certified in General Toxicology by the American Board of Toxicology.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21001-03 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Chemical Nephrotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/8	2/8	2/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Time- and dose-dependent effects of selected nephrotoxic agents on the ultrastructure and functional and biochemical status of target and non-target cells in the kidney are evaluated to study 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.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	William M. Kluwe	Supv. Pharmacologist	TRTP	NIEHS
Others:	Deepak K. Agarwal	Visiting Fellow	TRTP	NIEHS

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. These changes are compared to alterations in total organ (kidney) function.

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. Shortly thereafter, several tubular transport processes (e.g., ions, bulk fluids) are compromised. Functional recovery follows morphological evidence of repair.

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 molecular mechanisms of action. Also, the impact of age-related changes on nephrotoxic response to chemicals is being studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Kidney disease is a major cause of debilitation in the United States. Although 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	Report Writing
Benzaldehyde	Chronic
Diallylphthalate	Report Writing
Nitrofurantoin	Chronic
Nitrofurazone	Chronic
Bromobenzene	Prechronic
Diethylphthalate	Prechronic
Methylphenidate	Prechronic

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. 45: 3-10, 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. 45:129-133, 1982.

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.

Kluwe, W. M., Haseman, J. K., Douglas, J. F. and Huff, J. E.: The carcinogenicity of dietary di(2-ethylhexyl)phthalate (DEHP) in Fischer 344 rats and B6C3F$_1$ mice. J. Toxicol. Environ. Health 10: 797-815, 1982.

Kluwe, W. M., Montgomery, C. A., Giles, H. D. and Prejean, J. D.: Encephalopathy in rats and nephropathy in rats and mice after subchronic oral exposures to benzaldehyde. Food Chem. Toxicol. (in press), 1983.

Kluwe, W. M., Parker, G. A. and Manus, A. G.: Chronic toxicity of diallylphthalate in mice. Toxicol. Lett., (in press), 1983.

Kluwe, W. M., Haseman, J. K. and Huff, J. E.: The carcinogenicity of di(2-ethylhexyl)phthalate (DEHP) in perspective. J. Toxicol. Environ. Health (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21022-02 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunotoxic Chemicals Effect On Intermediary Metabolism of Mouse Lymphoid Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Michael P. Dieter Physiologist CTEB NIEHS

COOPERATING UNITS *(if any)*

Systemic Toxicology Branch

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 and tissue-specific pathway inhibition was correlated with functional defects in the immune system.

Principal Investigator and All Other Personnel Engaged on the Project:

Ralph Wilson	Technician	CPB	NIEHS
John E. French	Physiologist	CTEB	NIEHS
Michael P. Dieter	Principal Investigator	CTEB	NIEHS

PROJECT DESCRIPTION

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, spleen, lymph nodes, and liver.

There were specific immunotoxic and biochemical alterations in bone marrow, thymus, and spleen of mice treated with low concentrations of meruric chloride. The immunological defects were consistent with altered T-cell function as evidenced by decreases in both T-cell mitogen and mixed leukocyte responses. There was a particular association between the T-cell defects and inhibition of thymic pyruvate kinase, the rate limiting enzyme for glycolysis. Differences in the pattern of enzyme responses among these lymphoid organs implied that two mechanisms of mercury toxicity were operative - - one at high concentrations that caused physico-chemical enzyme inhibition and another at low concentrations that caused indirect enzyme inhibition through the pituitary-adrenal axis.

The estrogenic chemicals diethylstilbestrol, -estradiol and its metabolites also caused dose-related, specific inhibition of hexose monophosphate shunt enzymes in the bone marrow. Thymectomy experiments showed that inhibition of enzyme activity in bone marrow was mediated through the thymus at lower estrogen concentrations, while higher estradiol doses caused direct enzyme inhibition. Adrenalectomy or ovariectomy did not alter estrogenic inhibition of enzyme activity in bone marrow.

Individual classes of bone marrow cells were then separated into three bands on density gradients, yielding granulocyte-rich, lymphocyte-rich, and erythrocyte-rich cell fractions. Almost all of the DES inhibition of hexose monophosphate shunt enzyme activities (49%) could be attributed to the granulocyte-rich cell fraction. DES inhibited proliferation of granulocyte-macrophage stem cells two-fold more than multipotential stem cells. This project is now completed.

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.

PUBLICATIONS

Luster, M. I., Dean, J. H., Boorman, G. A., Dieter, M. P., and Hayes, H. T.: Immune Functions in Methyl and Ethyl Carbamate Treated Mice. Clin. Exp. Immunol. 50: , 1982.

Dieter, M. P., Luster, M. I., Boorman, G. A., Jameson, C. W., Dean, J. H., and Cox, J. W.: Immunological and Biochemical Responses in Mice Treated with Mercuric Chloride. Toxicol. Appl. Pharmacol. 68: , 1983.

Luster, M. I., Boorman, G. A., Korach, K. S., Dieter, M. P., and Hong, L.: Myelotoxicity Resulting from Exogenous Estrogens: Evidence for Bimodal Mechanism of Action. Clin. Exp. Immunol. 1983.

Boorman, G. A., Hong, H. L., Dieter, M. P., Hayes, H. T., Pohland, A. E., Stack, M., and Luster, M. I.: Myelotoxicity and Macrophage Alteration in Mice Exposed to Ochratoxin A. Toxicol. Appl. Pharmacol. 1983.

Dieter, M. P., Birnbaum, L. S., and Wilson, R.: Biochemistry of the Res in Aging F344 Rats. Mech. Aging Develop. 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21023-02 CTEB

PERIOD COVERED

October 1, 1983 to April 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Dimethyl Methyl Phosphonate on the Reproductive System of Male Rodents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

June K. Dunnick Chemist TRTP NIEHS

COOPERATING UNITS *(if any)*

Systemic Toxicology Branch

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.40	2.1 Technical

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dimethyl methyl phosphonate, a flame retardant and candidate nerve gas simulant, was tested for reproductive toxicity in male Fischer 344 rats and B6C3F$_1$ mice. Male rats and mice were dosed for 13 weeks at doses of 0, 500, 1000 and 2000 mg/kg and then mated to untreated females. Female rats and mice were sacrificed 14 days (rats) and 19 days (mice) after mating and pups examined. There was a dose related decrease in litter size and an increase in number of resorptions. Histologic abnormalities were seen in the testes of the high dose male rats. Histologic evaluation of the mouse tissues is still in progress.

Principal Investigator and All Other Personnel Engaged on the Project:

June K. Dunnick	Chemist	TRTP	NIEHS
Bhola N. Gupta	Pathologist	TRTP	NIEHS
Hank Solleveld	Pathologist	TRTP	NIEHS
James C. Lamb, IV	Research Biologist	TRTP	NIEHS
Martha Harris	Technical Supervisor	TRTP	NIEHS

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 and the male B6C3F_1 mouse.

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: Dimethyl methyl phosphonate (DMMP) a flame retardant and candidate nerve gas simulant was tested in the male Fischer 344 rat for reproductive toxicity. DMMP was administered to male rats by gavage for 90 days at doses of 0, 250, 500, 1000 and 2000 mg/kg. At day 84 the rats were mated to untreated female Fischer 344 rats. There was a dose related decrease in sperm count, sperm motility and the male fertility index. DMMP acted like a dominant lethal mutagen as demonstrated by an increase in the number of resorptions with increasing doses of the drug. The testes of the male rat were examined histologically to determine the relationship between reproductive function and pathologic abnormalities. DMMP altered reproductive function at all dose levels, while histologic abnormalities were seen only in the high dose group. Changes in the high dose group testes were characterized by lack of spermatogenesis or degeneration, vacuolization and necrosis of spermatogenic cells. A similar study to determine the reproductive toxicity of DMMP to the male B6C3F_1 mouse is underway.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dimethyl methyl phosphonate (DMMP) is used as a flame retardant and has been considered for use as a nerve gas simulant in experimental situations by the U.S. Armed Forces. 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21030-01 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemically-Induced and Spontaneous Mononuclear Cell Leukemia in Fischer 344 Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John Edgar French Physiologist CTEB NTP/NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch
Systemic Toxicology Branch
Cellular and Genetic Toxicology Branch

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch, TRTP

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A spontaneous mononuclear cell leukemia of Fischer 344 rats is the principal cause of death for up to 50% of the rats dying between 18-26 months of age. A high incidence of this leukemia has been diagnosed in both control and treated rats in two-year chronic toxicity tests. Understanding the variation in the chemically-induced incidence of leukemia is complicated by the high spontaneous occurrence of this disease in the 18-24 month old Fischer 344 rat. Responses to chemical treatment range from statistical significance increases to both positive and negative trends with respect to dose. Other observations also demonstrate the complexity of the biology of this disease. In male rats only, a decreased incidence of leukemia was observed in corn oil gavaged controls compared to dosed-feed controls. An inverse relationship between incidence of Fischer rat leukemia and hepatocellular tumors has also been described. The Fischer 344 rat mononuclear leukemia is incompletely defined and characterized.

The long range goal of this research project is to clearly define the spontaneous leukemia and chemically-induced leukemia and their associated pathophysiology in order to clarify the interpretation of chemical toxicity in those chronic tests where the hematopoietic system may be a target organ.

Principal Investigator and All Other Personnel Engaged on the Project:

John E. French	Physiologist	CTEB	NIEHS
Michael P. Dieter	Physiologist	CTEB	NIEHS
Robert M. Maronpot	Pathologist	CPB	NIEHS
Gary A. Boorman	Pathologist	CPB	NIEHS
Linda Birnbaum	Pharmacologist	STB	NIEHS
Pat Blair	Medical Technologist	CPB	NIEHS
Joe Haseman	Statistician	SBB	NIEHS
Lilie Hong	Technician	CPB	NIEHS
Richard Irwin	Biophysicist	CTEB	NIEHS
William Jameson	Supervisory Chemist	PRB	NIEHS
Michael Luster	Immunologist	STB	NIEHS
Fred Talley	Supervisory Technologist	CPB	NIEHS
Raymond Tennant	Supervisory Microbiologist	CGTB	NIEHS
Ralph Wilson	Biological Lab Technician	CPB	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

The results of the preliminary investigations indicate that this rat leukemia presents in NTP chronic toxicity tests as described in the literature, confirming the diagnosis of mononuclear cell leukemia. We have obtained additional information describing its pathology, cytology (light and ultrastructural), and malignant cell biochemistry. In addition, basic information was obtained on the cell biology and transplantation characteristics of the malignant mononuclear cell to facilitate detailed studies on the further characterization and determination of the origin and pathophysiology of this leukemia and its impact upon NTP chronic toxicity studies.

A. It is our proposed course to complete basic investigations on the characterization of normal and leukemic mononuclear cells from F344 rat hematopoietic tissue and peripheral blood, which have not been previously described.

   (1) Define the optimal cell separation and isolation procedures using linear and discontinuous density gradients.

   (2) Determine the heterogeneity of normal and leukemic cell populations according to their cellular bouyant density, morphology (light and EM), cytochemistry and cellular biochemistry.

   (3) Use service contracts available for determining the karyotype and chromosomal analysis of isolated normal and leukemic mononuclear cells using Giemsa, quinacridine, and bromodeoxyuridine fluorescent techniques.

(4) Use service contracts or collaborative contacts available for performing an initial screen for the presence of retrovirus and/or retroviral oncogenes in the normal and leukemic mononuclear cell.

B. To compare the F344 rat hematopoietic tissue and peripheral blood cells and leukemic mononuclear cells according to their cell surface antigens, receptors and cytochemistry.

(1) Develop and/or adapt procedures for determining rat cell surface antigens and receptors using fluorescent and peroxidase conjugated antibody diagnostic techniques (using fluorescence microscopy and flow cytometry when available) and conventional receptor determination methodology.

(2) Correlate cell surface antigen and receptor data to known cytochemical, morphological and cell biochemistry data for aid in determining leukemic cell origin and functional lineage.

C. To complete and further define the requirements for leukemic cell transplantation and characterize the morphologic features of the disease in the recepients.

(1) Determine the proliferation kinetics, organ invasion, and survival after transplantation of 10^2 to 10^8 mononuclear leukemic cells.

(2) Describe the morphologic and selected pathophysiologic features during the course of the disease.

(3) Determine the cellular biochemical change in hematopoietic tissue and periperal blood mononuclear cells during the time course of organ invasion as a possible diagnostic tool of pre- to early leukemic state. Correlate biochemical changes with morphological, cytochemical, and clinical chemistry over the same time period.

D. Define the diagnostic criteria for early and late cases of this leukemia for NTP pathologists based upon the information obtained to date.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The investigation of F344 rat mononuclear cell leukemia affords the unique opportunity to investigate a spontaneously occurring tumor and its origin, and develop clonally derived cell line(s) for in vitro study of this disease and develop an animal model for experimental chemical induction of leukemia for toxicity and test methods development. In addition, investigation of the impact of this leukemia upon the interpretation of chronic toxicity results of NTP tests is of significant importance to the Institute's program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21061-01 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between Chemical Dose and Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/8	2/8	2/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several organohalide compounds are metabolized to reactive intermediates presumed to be the ultimately toxic molecules. The reactive metabolites are detoxified by reacting with tissue non-protein sulfhydryls (NPS), and acute toxicity occurs only when NPS have been depleted below a critical level. Upon prolonged chemical exposure, however, a dynamic state exists between chemical metabolism, NPS depletion, NPS synthesis, and lesion development. Whether or not the same relationship exists between tissue NPS concentrations and the development of lesions in a chronic exposure situation as in an acute one is being evaluated.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	William M. Kluwe	Supv. Pharmacologist	TRTP	NIEHS
Others:	Deepak K. Agarwal	Visiting Fellow	TRTP	NIEHS
	Robert Maronpot	Pathologist	TRTP	NIEHS
	Jerry Hardisty	Pathologist	Environmental Pathology Laboratories, Raleigh, North Carolina	

PROJECT DESCRIPTION

METHODS EMPLOYED: Rodents are treated for 1, 5, 10 or ~ 60 days with one of several organohalide compounds (e.g., bromobenzene, vinyl chloride, ethylene dibromide) by gavage or inhalation. At representative times post-exposure, the animals are killed and tissues are evaluated for morphological changes and for NPS contents and other biochemical parameters. In some instances, the time-dependent dispositions and distributions of the test chemicals are monitored.

MAJOR FINDINGS AND PROPOSED COURSE: A single exposure to bromobenzene produces a dose-dependent depletion of hepatic NPS followed by a "rebound" to higher than normal levels. Hepatotoxicity occurs only when hepatic NPS are depleted below 40% of normal. Following multiple exposures, a higher steady-state concentration of hepatic NPS is achieved. Although bromobenzene still depletes hepatic NPS, the NPS concentration apparently does not fall below a "critical" level and hepatotoxicity does not develop. The mechanism and limits of this protective change in response to prolonged bromobenzene exposure are currently being studied.

Organohalides supplied by inhalation have a lower propensity for depleting hepatic NPS, but a greater propensity for depleting non-liver NPS (e.g., lung). However, animals exposed multiply still tend to exhibit lesser toxic changes in tissue morphology than those exposed singly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: A better understanding of the dynamic nature of dose-response relative to NPS depletion in chronic studies will enhance our ability to properly choose doses for chronic studies. Similarly, exploration of the interrelationships between dose, NPS and injury in chronic exposure situations may help clarify the appropriateness (or inappropriateness) of high dose to low dose extrapolations for chronic studies involving chemicals exhibiting non-linear pharmacokinetics.

PUBLICATIONS

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21062-01 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Phthalate Ester Toxicities in Mammalian Species

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-4/8	7/8	5/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phthalate esters are plasticizers incorporated into nearly all plastic materials. The biochemical and ultrastructural effects of di(2-ethylhexyl)phthalate (DEHP) and related chemicals on the rodent liver are being studied in order to assess potential mechanisms of phthalate ester hepatocarcinogenicity.

DEHP is also a male chemosterilant and is teratogenic in mice. Studies are being conducted to determine the role of zinc in the pathophysiology of these reproductive effects and to discern no-observed toxic effect levels.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	William M. Kluwe	Supv. Pharmacologist	TRTP	NIEHS
Others:	Deepak K. Agarwal	Visiting Fellow	TRTP	NIEHS
	James C. Lamb, IV	Biologist	TRTP	NIEHS
	Robert Maronpot	Pathologist	TRTP	NIEHS
	Scott Eustis	Pathologist	TRTP	NIEHS
	Hank Solleveld	Pathologist	TRTP	NIEHS
	Ronald Melnick	Biochemist	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: For the hepatic effects studies, rodents treated with DEHP are analyzed for evidence of oxidative stress to the liver and other organs, and dose-dependent effects on intermediary metabolism, toxic response and serum lipid concentrations are compared.

For the reproductive studies, male rats treated with DEHP are mated to determine fertility, and assessments are made of male gonadal structure, function and biochemistry. The role of dietary and tissue zinc in enhancing or ameliorating the gonadal effects of DEHP are monitored.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of low dietary zinc and DEHP on the weights, morphologies and enzymatic content of the testis and accessory sex glands are similar. The effects of DEHP on all of these can be blocked by high dietary zinc concentrations, suggesting a causal role of zinc depletion in the pathogenesis of DEHP-induced testicular injury.

Butyl benzyl phthalate causes testicular atrophy in male rats accompanied by a decreased serum concentration of testosterone (FSH and LH increased). This compound also reduced the cellularity of the bone marrow after 14 days of treatment.

Subchronic studies are currently being conducted with butyl benzyl phthalate or DEHP, the latter employing varying concentrations of dietary zinc. The butyl benzyl phthalate study in rats will ultimately include a 2 year chronic exposure experiment. Comparative studies on the hepatic effects of DEHP, di(2-ethylhexyl)-adipate and similar compounds will be conducted in parallel with chronic bioassays for carcinogenic activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Phthalate ester plasticizers are ubiquitous contaminants of the general environment, and leachable components of most plastic consumer products. The frequent exposure of humans to these substances and the serious toxic effects (e.g., carcinogenesis, teratogenesis, sterility) associated with their prolonged use in rodents necessitates a clear understanding of their toxic potential. This task is best accomplished through an integrated program of general testing for toxicity and elucidation of mechanisms for specific toxic effects.

PUBLICATIONS

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21063-01 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tests for the Detection and Monitoring of Chemical-Induced Pulmonary Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Rajendra S. Chhabra Supv. Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

Pulmonary Pathology Group, LPFT

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch, TRTP

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many chemical agents are capable of damaging the lungs. However, the potential for pulmonary injury by such agents is not at present predictable and the detection of injury requires the development of lesions capable of identification by histopathological procedures. The alveoli and distal airways of the lungs are covered with a very thin film of fluid known as the pulmonary extracellular lining (EL) which may be sampled without excessive danger or discomfort to patient or subject. Since the EL is in intimate contact with the alveolar and bronchial epithelium it is proposed that chemical agents could damage and release epithelial components into the EL. The objectives of this research are the development of biochemical tests for the assessment of the pulmonary toxicity of chemical agents by quantitative measurements of epithelial cell components in the EL of laboratory animals exposed to various classes of pulmonary toxicants.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	Rajendra S. Chhabra	Supv. Pharmacologist	TRTP	NIEHS
Others:	Kimeri D. Collins	Biological Lab Tech.	TRTP	NIEHS
	Gary E.R. Hook	Research Chemist	LPFT	NIEHS
	Paul Nettesheim	Chief	LPFT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The pulmonary extracellular lining of rats and rabbits is sampled by lavaging the lungs with isotonic saline via the trachea.

MAJOR FINDINGS AND PROPOSED COURSE: Lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (GDH) were investigated as potential markers for the detection of cellular damage in the lungs. Both enzymes have been reported as components of the soluble phase of cellular injury. Both LDH and GDH were highly active in the lungs of rabbits (LDH, 630 $\pm$ 80 [4] mmoles/NADH oxidized/min/pair of lungs; GDH, 102 $\pm$ 51 [4] µmoles/NADH oxidized/min/pair of lungs). However, the activities of these two enzymes were not stable. GDH activity declined from 34.0 $\pm$ 17.1 (4)[mmoles/min/mg protein] in the soluble phase of lung homogenates to 14.1 $\pm$ 14.6 (4) within 24 hours when stored in the frozen state. LDH showed similar instabilities although storage of the enzyme at room temperature reduced the rate at which the LDH disappeared. The disappearance of GDH was not reduced by storage of the enzyme at room temperature. LDH and GDH consisted of 5 and 3 major isozymes, respectively, as indicated by polyacrylamide gel electrophoresis. The stability of the isozymes were not similar since the number of bands and intensity of staining changed with time of storage. Stabilization of LDH was achieved by the inclusion of HEPES (50 mM) in the medium. Storage of LDH at -15°C without HEPES resulted in activity falling from 1.06 $\pm$ 0.22 (µmoles/min/mg soluble phase protein) to 0.49 $\pm$ 0.16 over a 7 day period. In the presence of HEPES the activity of LDH after 7 days of storage at -15°C was 1.11 $\pm$ 0.41. GDH was not stabilized by HEPES. These results indicate that neither LDH nor GDH may be suitable as markers for the detection of pulmonary damage unless measured immediately or stored in the presence of a stabilizing agent. In addition, previous work of other laboratories reporting the release of LDH into the EL in response to lung injury with particulate materials may require re-evaluation in view of the instability of the enzymes observed in the present studies. Future work will examine the release of LDH and other potential markers of lung injury into the pulmonary extracellular lining in response to treatment of animals with pneumo-toxic chemicals such as naphthalene and carbon tetrachloride.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: The lungs are a target of many toxic chemical agents. However, sensitive biochemical methods for the detection and monitoring pulmonary damage and disease induced by such agents are not currently available. This research is aimed at the development of methods for the early detection of lung injury as a result of chemical exposure.

PUBLICATIONS

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-30100-04 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toxic Effects of 1,2-Dibromo-3-chloropropane on the Urogenital System

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6/8	2/8	4/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 and development 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 does DBCP.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	William M. Kluwe	Supv. Pharmacologist	TRTP	NIEHS
Others:	James C. Lamb, IV	Biologist	TRTP	NIEHS
	Deepak K. Agarwal	Visiting Fellow	TRTP	NIEHS
	Robert Maronpot	Pathologist	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Toxic effects are being studied in developing or mature 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 β-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, although 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, but neonates are extremely sensitive to the gonadotoxic 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. The decreased fertility appears to occur secondary to a decrease in sperm energy metabolism. 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 the extreme sensitivity of very young animals to DBCP gonadal 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 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.

Kluwe, W. M.: Chemical modulation of 1,2-dibromo-3-chloropropane toxicity. Toxicol. (in press), 1983.

Kluwe, W. M.: Effects of partial hepatectomy on organ-specific toxic response to 1,2-dibromo-3-chloropropane. J. Appl. Toxicol. (in press), 1983.

Kluwe, W. M., Gupta, B. N. and Lamb, J. C., IV.: The comparative effects of 1,2-dibromo-3-chloropropane (DBCP) and its metabolites, 3-chloro-1,2-propaneoxide (epichlorohydrin), 3-chloro-1,2-propanediol (alphachlorohydrin) and oxalic acid on the urogenital system in male rats. Toxicol. Appl. Pharmacol. (in press), 1983.

Kluwe, W. M., Lamb, J. C., IV, Greenwell, A. and Harrington, F. W.: 1,2-Dibromo-3-chloropropane (DBCP)-induced infertility in male rats mediated by a post-testicular effect. Toxicol. Appl. Pharmacol. (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-30101-04 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renal Function Tests as Indicators of Nephrotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/8	1/8	1/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.: William M. Kluwe Supv. Pharmacologist TRTP NIEHS

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 the chemical treatments.

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. Also, the toxicologic/functional significance of chemically-induced anisokaryosis and cytomegally in the kidney are being evaluated.

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 9: 619-635, 1982.

Kluwe, W. M.: Developed resistance to mercuric chloride: failure to protect against other nephrotoxicants. Toxicol. Lett. 12: 19-25, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30102-04 CTEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between Halogenated Aliphatic Chemicals & Renal Tubular Cells In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William M. Kluwe Supervisory Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4/8	2/8	2/8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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, potentially nephrotoxic chemicals) is manipulated to suggest biochemical mechanisms of action.

Principal Investigator and All Other Personnel Engaged on the Project:

P.I.:	William M. Kluwe	Supv. Pharmacologist	TRTP	NIEHS
Others:	Deepak K. Agarwal	Visiting Fellow	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparations of renal tissue (e.g., slices, explants, 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 sources of renal tissue are rat, mouse, hamster, guinea pig and rabbit.

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. Emphasis will be placed on interference with cellular energy metabolism and other molecular mechanisms of toxicity.

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.), Plenum Publishing Corp., New York, pp. 331-344, 1982.

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. 220: 597-603, 1982.

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 UVA light. This project will test a variety of promising psoralen compounds and compare the relative toxicities. The toxicity of 8-methozypsoralen (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: UVA light exposure.

MAJOR FINDINGS AND PROPOSED COURSE: This contractor has completed a 13-week study in the HRA/Skh mouse comparing the toxicologic properties of 8-methoxypsoralen (8-MOP), 3-carbethoxypsoralen (3-CEP), 5-methylisopsoralen (5-MIP), and 5-methoxypsoralen (5-MOP) with and without UVA light. The psoralen was delivered by a "pulsed feed" technique which minimized topical contact with the drug, and allowed for a controlled UVA light exposure 30 minutes after drug intake. 8-MOP and 5-MOP showed severe photobiologic skin toxicity when used in combination with UVA. Little or no photobiologic skin toxicity was seen with 3-carbethoxypsoralen and 5-methylisopsoralen. There was no skin response to drug in the absence of UVA. A complete histopathologic study is in progress on the tissues from the 13-week study. A one year study with 8-MOP and UVA is now under way to determine tumor response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PUVA therapy (8-MOP plus UVA light) was approved in May 1982 by the Food and Drug Administration for the treatment of psoriasis. It is estimated that approximately 3% of the United States population suffers from this disease. The NIADDK Collaboration Psoralen Study Group has found that there are three possible complications from PUVA treatment; increase in skin cancer; eye lesions; and immunologic damage. Thus the object of the NTP animal studies is to help identify the toxic properties of PUVA therapy. 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 - The tissue distribution and excretion patterns of three uniformly labelled ^{3}H-psoralens, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 3-carbethoxypsoralen (3-CEP), were investigated in hairless HRA/Skh mice with and without UV-A light (3-9 J/cm^2). Female mice were given a single oral dose of 6 mg/kg of the ^{3}H-psoralen corn oil in a 96-hr tissue distribution and excretion study. Each psoralen was rapidly absorbed through the gastrointestinal (GI) tract. The highest ^{3}H levels occurred in GI tract, skin, blood and liver; the lowest was observed in eyes. 82-88% of administered ^{3}H was recovered in the urine, feces and respiration products in the 96 hours after administration of 8-MOP, 5-MOP or 3-CEP. In animals treated with 8-MOP or 5-MOP, over 25% of ^{3}H was excreted as $^{3}H_2O$, only 9% was excreted as $^{3}H_2O$, in 3-CEP treated animals. Maximum phototoxicity resulted 1½ hrs after a 30 minute exposure to UV-A light, at which time the skin content of ^{3}H was elevated in 8-MOP or 5-MOP treated animals. This correlation was not evident with 3-CEP. The urinary metabolic profiles for these compounds in HRA/Skh mice are currently being investigated by high performance liquid chromatography. Genetics studies - This contract 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: PUVA therapy (8-MOP plus UVA light) was approved in May 1982 by the Food and Drug Administration for the treatment of psoriasis. It is estimated that approximately 3% of the United States population suffers from this disease. The NIADDK Collaborative Psoralen Study Group has found that there are three possible complications from PUVA treatment; increase in skin cancer; eye lesions; and immunologic damage. The object of this project is to study the tissue distribution patterns of the psoralens and metabolic products, and to correlate the metabolism of the drug with skin toxicity. This project also seeks to correlate genetic damage from PUVA treatment with tumor formation. 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.

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: $49,368

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, 1983 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 tremolite and crocidolite and amosite asbestos studies were presented to and were approved by the NTP Board of Scientific Counselors in FY 1983. No toxic or neoplastic effects were recognized for any of these three materials. The chrysotile studies will be reported in late FY 1983.

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.

PUBLICATIONS

McConnell, E.E.: NTP technical report on the carcinogenesis bioassay of amosite asbestos. NTP 82-86, in press.

END

McConnell, E.E.: NTP technical report on the carcinogenesis bioassay of crocidolite asbestos. NTP 83-030, in press.

McConnell, E.E.: NTP technical report on the carcinogenesis bioassay of tremolite. NTP 82-95, in press.

McConnell, E.E., Rutter, E.E., Ulland, B., and Moore, J.A.: Carcinogenesis bioassay of amosite asbestos and tremolite in F344 rats. Environ. Health Perspectives, 1983, in press.

McConnell, E.E., Shefner, A.M., Rust, J., and Moore, J.A.: Carcinogenesis bioassay of amosite and chrysotile asbestos in Syrian golden hamsters. Environ. Health Perspectives, 1983, in press.

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
MND	———	MTD
	———	1/3 MTD
	———	No treatment
1/3 MND	———	1/3 MTD
1/10 MND	———	1/10 MTD
untreated	———	MTD
	———	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 femal 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: The chronic phase of this study is underway.

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 were extended to 9 weeks for this compound to determine if the carcinogenic potential of this chemical can be detected at an early age. The chronic phase of this study is underway.

Firemaster FF-1: The MND studies have been completed. The chronic phase on this chemical is underway.

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.

Chin, A. E., Kurtz, P. J., Carlton, B. D., Peters, A. C. and Chhabra, R. S.: The disposition and metabolism of ethylenethiourea in maternal, fetal and neonatal tissues after perinatal exposure of rat and mouse dams. The Toxicologist, 3: 635, 1983.

CELLULAR AND GENETIC TOXICOLOGY BRANCH

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The major goal of the Cellular and Genetic Toxicology (CGT) Branch is to provide an integrated research and testing program using a variety of test systems to evaluate the genetic toxicity of chemicals to which humans are exposed. The program is also aimed at developing an understanding of the mechanisms of cellular and genetic toxicity to provide a better basis for further development of test systems and interpretation of test results. Emphasis is currently placed on tests that measure mutagenicity and aneuploidy in microbial cells as well as mutagenicity, DNA damage, and neoplastic transformation in mammalian cells. Test data will be used to select chemicals and to set priorities for further testing, and to aid 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 that can be used to predict carcinogenicity and germ cell mutagenicity of chemicals, and, thereby, reduce the need for in vivo assays, or to assist in setting testing priorities for long-term animal assays. However, for short-term tests to be predictive, several criteria must be fulfilled. These include a knowledge of the reproducibility of individual test results, the accuracy with which test results from a single or multiple tests predict a toxic effect in vivo, and the relationship of the endpont measured to carcinogenicity, mutagenicity, or other manifestations of in vivo toxicity. The application of a group of complementary tests that meet these criteria may 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 establish the accuracy with which we can predict carcinogenic effects in animals. Even with the appropriate use of available test systems, some potential carcinogens (or cocarciongens or tumor promotors) may not be identified, particlarly those that do not induce damage leading to observable gene mutations or chromosomal changes. Therefore, it is important that we continue to develop new methods that are capable of detecting carcinogens that are not identified by the assays currently in use and of identifying chemicals that "promote" tumor development. In order to accomplish these goals, it is important that the program remain involved in, and responsive to, basic research developments.

A substantial portion of Branch resources are committed to studies of chemically-induced mutations. These mutation studies can be divided into two categories: somatic cell and germ cell. The major difference between the two is that mutations arising in germ cells can be transmitted to subsequent generations, while somatic cell mutations are expressed only in the affected individual. Germ cell mutagenicity test systems are considered relevant for somatic mutation because they measure mutagenicity in mitotically dividing cells. However, the information gained from tests using germ cell assay systems also has implications for heritable mutation risk because a chemical that is mutagenic in vitro has the potential to be mutagenic in germ cells. By the same argument, germ cell mutagens are likely to be somatic cell mutagens.

The portion of the program concerned with validation of assays and the testing of chemicals is performed primarily through extramural contracts and interagency agreements; however, basic research, the development or modification of tests, and the management and analysis of data are generally performed within NIEHS.

The key extramural contract activities include: mutagenesis testing in Salmonella, Drosophila, and mammalian cells; chromosome aberrations (CAs) and sister chromatid exchanges (SCEs); the development and evaluation of an in vivo assay to detect 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; an in vitro assay in mammalian cells for induced DNA damage; compound an 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 using coded compounds in at least two laboratories. (The latter two systems measure chemical enhancement of viral transformation or viral mediated transformation).

A coordinated effort was initiated in FY 1981 to assess the genetic toxicity of chemicals that are likely candidates for rodent bioassay. A second objective of the coordinated testing effort is to acquire test results from several short-term tests on a group of carcinogens and, in particular, noncarcinogens in order to better determine the predictve capabilites of individual and multiple tests. Other projects include an effort to develop a standardized protocol by which the frequencies of CA's and SCE's 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. The measurement of chemically-induced germ cell mutations in mice is done by both the morphological and biochemical specific-locus assays. Other developmental projects include analysis of mutagens produced in cooked foods; and an attempt to develop an assay for chemically-induced transpositions of specific DNA sequences in Drosophila.

Intramural research efforts involve both prokaryotes and eukaryotes. Studies using the Salmonella typhimurium strains include attempts to increase our understanding of the Salmonella mutagenicity 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.

The role of DNA repair mechanisms during meiotic development is being examined in yeast. We have identified genes for the repair of DNA damage in mitotic cells which become essential in germinal processses. These repair genes are being examined for their mode for action during meiotic DNA metabolism, particularly recombination, and for their gene products. Of particular interest is the RAD52 gene which is involved in DNA double-strand break repair and meitoic recombination. It controls a nuclease which has now been purified to homogeneity. Other genes essential to meiotic development are also being characterized and cloned to determine their specific roles in meiosis.

Mutation rates are under genetic control. We are examining the extent to which DNA repair mechanisms control the frequencies and types of mutagenic events in

Drosophila melanogaster. Emphasis has been placed on the genetic characterization of an X-ray-dependent mutator that is unable to repair chromosome breaks.

The Mammalian Cell Molecular Genetics Group has been analyzing potential DNA sequence transpositions and alterations in gene structure in the radiogenic myeloid leukemia of the RFM/Un mouse. Analysis of DNA by restriction enzyme digestion and hybridization with specific cloned DNA probes has revealed a correlation between both myeloid leukemia and reticulum cell sarcoma and increased copy number of the endogenous ecotropic provirus. Recombinant DNA cloning of the tumor specific provirus integration sites has been initiated. Future plans include comparison of these sites in individual tumors to determine if common integration sites or domain exists. The proximity of the relocated provirus to known oncogenes, and the effect of the integration on gene expression in the region will be examined.

The problem of organ and species specificity of chemical carcinogens is being studied by measuring the production of metabolites, from primary epitheilal cells (activator cells) of liver, lung and urinary bladder of different species, which induce genetic toxicity. The activator cells are cocultivated with target cells (V79 hamster cells or Salmonella) to measure toxicity, SCE's and mutation. Future studies will involve activation of aromatic amines by measuring metabolite production and DNA-adduct formation and repair. Also, the role of prostaglandin synthetase, an enzyme known to contribute to carcinogen activation will be studied. Long range plans include development of systems to measure chemically-induced multiple genetic endpoints in human cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21012-02 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organ and Species Specificities in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

R. Langenbach Microbiologist CGTB NIEHS

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, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 usd 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.

Principal Investigator and All Other Personnel Engaged on the Project:
R. Langenbach Microbiologist CGTB NIEHS

PROJECT DESCRIPTION

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 mechanisms of specificity in cancer initiation and hopefully, with the proposed use of human cells, will facilitate extrapolation of bioassay data to human hazard.

PUBLICATIONS

1. Kuszynski, C., Somogyi, A., and Langenbach, R.: Modification of benzo(a)-pyrene-induced transformation of C3H/10T½ cells by pregnenolene-16a-carbonitrile and dexamethasone. Cancer Letters 15: 215-221, 1982.

2. Allen, J. W., Langenbach, R., Nesnow, S., Sasseville, L., Leavitt, S., Campbell, J., and Sharief, Y.: Comparative genotoxicity studies of ethyl-carbamate and related chemicals: further support for vinyl carbamate as a proximate carcinogenic metabolite. Carcinogenesis 1437-1441, Vol. 3 No. 12, 1982.

3. Langenbach, R., and Nesnow, S.: Cell-Mediated Mutagenesis, an in vitro Approach to Study Organ Specificity of Chemical Carcinogens. In Homburger, F. (Ed.): Safety Evaluation and Regulation of Chemicals. New York, Karger, 1983, pp. 142-150.

4. Langenbach, R., and Oglesby, L.: The Use of Intact Cellular Activation Systems in Genetic Toxicology Assays. In de Serres, F.J., and Hollaender, A. (Ed.): Chemical Mutagens - Principles and Methods for Their Detection. New York, Plenum Press (in press) 1983.

5. Langenbach, R., and Nesnow, S.: Cell-Mediated Mutagenesis, An Approach to Studying Organ Specificity of Chemical Carcinogens. In Langenbach, R., Nesnow, S., and Rice, J. M., (Eds.): Organ and Species Specificity in Chemical Carcinogenesis. New York, Plenum Press, 1983, pp. 377-390.

6. Allen, J. W., Sharief, Y., Langenbach, R., and Waters, M. D.: Tissue-Specific Sister Chromatid Exchange Analyses in Mutagen-Carcinogen Exposed Animals. In Langenbach, R., Nesnow, S., and Rice, J. M., (Eds.).: Organ and Species Specificity in Chemical Carcinogenesis. New York, Plenum Press, 1983, pp. 451-472.

7. Langenbach, R., Nesnow, S., and Rice, J. M., (Eds.): Organ and Species Specificity in Chemical Carcinogenesis. New York, Plenum Press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21013-02 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Lawrence R. Boone Senior Staff Fellow 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:
2.9	1.2	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Analysis of spleen DNA of RFM animals with myeloid leukemias and reticulum cell sarcomas has revealed copies of endogenous provirus at new locations in the mouse genome. Replication of the RFM endogenous ecotropic viruses is strongly restricted and the novel integration identified may be due to an intracellular transposition mechanism. Fragment exchange experiments have been done with infectious endogenous and exogenous retroviral genomes to determine the mechanism by which the RFM mouse restricts the endogenous provirus. The results indicate an involvement of the viral gag gene region, possibly by an Fv-1 mechanism.

Principal Investigator and All Other Personnel Engaged on the Project:

Lawrence R. Boone	Senior Staff Fellow	CGTB	NIEHS
Raymond W. Tennant	Supervisory Microbiologist	CGTB	NIEHS
Paul L. Glover	Biological Lab Technician	CGTB	NIEHS
Cynthia L. Innes	Biological Lab Technician	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Recombinant DNA techniques have been used to generate molecular probes for specific regions of the endogenous ecotropic retrovirus genome of RFM mouse. Restriction endonuclease analysis of spleen DNA from normal and leukemic animals was performed with these specific probes. Molecular clones of germ line and transposed proviral genomes have been isolated from an RFM myeloid leukemia. In vitro constructed recombinants of endogenous and exogenous retroviral genomes have been made and analyzed to study the mechanism of RFM endogenous virus restriction.

MAJOR FINDINGS AND PROPOSED COURSE: Analysis of spleen DNA from RFM mice with myeloid leukemia or reticulum cell sarcomas has revealed a correlation between these diseases and the presence of additional copies of the endogenous ecotropic provirus at new integration sites. The proposed course is a more detailed analysis of these additional provirus copies to determine if a common genomic domain is involved. Such a genomic domain may contain a specific oncogene which is effected by proviral integration. Restriction enzyme analysis will continue using the molecular clones of germ line and myeloid leukemia-derived proviruses and specific probes to detect flanking cellular regions will be constructed to probe other primary leukemias. Gene expression of regions flanking integrated provirus will be examined in normal and neoplastic cells. Proviruses from other primary myeloid leukemias and reticulum cell sarcomas will also be cloned and analyzed. The movement of this proviral genetic element by the typical retrovirus replication cycle is strongly restricted. Infectivity analysis of in vitro constructed recombinant viral genomes indicated that the gag gene region of the viral genome is involved in this restriction. This may represent an Fv-1 like mechanism and additional in vitro recombinants will be made to examine this question.

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/NIEHS. This project is aimed at providing basic information concerning those processes and on which reliable assay systems can be based.

Boone, L.R., Myer, F.E., Yang, D.M., Kiggans, J.O., Koh, C., Tennant, R.W., and Yang, W.K.: Analysis of recombinant DNA clones of the endogenous Balb/c murine leukemia virus WN18Q2N: Variation in long terminal repeat length. J. Virol. 45: 484-488, 1983.

Liou, R.S., Boone, L.R., Kiggans, J.O., Yang, D.M., Wang, T.W., Tennant, R.W., and Yang, W.K.: Molecular cloning and analysis of the endogenous retrovirus chemically-induced from RFM/Un mouse cell cultures. J. Virol. 46: 288-292, 1983.

Boone, L.R., Myer, F.E., Yang, D.M., Kiggans, J.O., Koh, C., Tennant, R.W., and Yang, W.K. Variation of LTR size in molecular clones of the Balb/c endogenous ecotropic murine leukemia virus. Progr. Nucl. Acid Res. Mol. Biol. 29: 205-213, 1983.

Tennant, R.W., Boone, L.R., Lalley, P., and Yang, W.K.: Endogenous retrovirus and radiation-induced leukemia in the RFM mice. Progr. Nucl. Acid Res. Mol. Biol. 29: 75-86, 1983.

Yang, W.K., Boone, L.R., Tennant, R.W., and Brown, A.: Fv-1 restriction of murine leukemia viruses: a model for studying host genetic control of retroviral gene movement and leukemogenesis. Progr. Nucl. Acid Res. Mol. Biol. 29: 175-192, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21014-02 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mathematical Modeling of DNA Repair and Recombination in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

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:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 mathematical simulation 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 and to relate exchanges to models for recombination. Biological results from E coli, yeast and mammalian cells are being evaluated using the mathematical simulation.

Principal Investigator and All Other Personnel Engaged on the Project:

M. Resnick	Research Geneticist	CGTB	NIEHS
T. Darden	Staff Fellow	BRAP	NIEHS

PROJECT DESCRIPTION

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 simulate 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 normal meiotic molecular exchanges in yeast. Therefore, DNA damage at large distances from sites of exchange can modify normal recombination. These results are supported by genetic evidence. 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 if the mode of post-replication repair is via a single Holiday exchange.

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.

PUBLICATIONS

Darden, T., and Resnick, M.A.: The relationship between DNA damage and recombination: modeling and analyses of sucrose gradient sedimentation patterns. J. Cellular Biochemistry 7B: 233, 1983.

Darden, T.A., and Resnick, M.A.: A mathematical analysis of recombination as measured by surcrose gradients. J. Appl. Probability (Submitted)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21015-02 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Synthesis on Damaged Templates by Yeast Crude Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

P. Moore I.P.A. CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used an in vitro approach to investigate the ability of UV induced pyrimidine dimers to block DNA replication in yeast. Our previous in vivo work had indicated that DNA synthesized following UV irradiation of mitotic or meiotic yeast was larger than the interdimer distance suggesting the possibility of trans-dimer synthesis.

Using a specially constructed double stranded ϕX174 DNA template, we have studied semi-crude protein extracts that are capable of replicating yeast DNA. By sequencing the products of synthesis we can determine whether synthesis terminates at (or bypasses) pyrimidine dimers. The results indicate that extracts from normal and UV irradiated mitotic, as well as meiotic, cells terminate at dimers, and no evidence has been found for bypass.

Principal Investigator and All Other Personnel Engaged on the Project:

P. Moore	I.P.A.	CGTB	NIEHS
M. Resnick	Research Geneticist	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Various repair-deficient mutants of Saccharomyces cerevisiae are genetically manipulated and grown using standard techniques. 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 techiques for ϕX174 have been described by Moore and Strauss [Nature 278 (1969) 664]. The mutagen treated ϕX174 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: We have designed and constructed a DNA template with which it is possible to assay for lesion-bypass in a single stage reaction. This template is utilized efficiently by the yeast extract system. We have observed termination of replication at pyrimidine dimers in our yeast extracts and can compare this with synthesis carried out by purified E. coli DNA polymerase I under conditions that do not allow bypass.

Extracts have been prepared from normal (log phase) cells, cells pretreated with UV light and from cells undergoing meiotic DNA replication. At this stage of the analysis, there is no evidence for bypass by any of the extracts; however, a more quantitative analysis of the data will allow more certainty to this conclusion. We are investigating the extent to which these results are due to the biological absence of bypass or deficiencies in the extract replication system. We hope to achieve "positive control" with a purified enzyme system in order to resolve these ambiguities.

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.

PUBLICATIONS

Moore, P. D., and Resnick, M. A.: Replication of UV irradiated DNA in yeast crude extracts. J. Cell. Biochem. Supp 7B: 221, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21016-02 CGTB

PERIOD COVERED: October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Enzymes Involved in DNA Repair and Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Terry Chow Visiting Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH: Cellular and Genetic Toxicology Branch

SECTION:

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. The rad52 mutant of Saccharoymces cerevisiae was shown to be deficient in an alkaline deoxyribonuclease by the use of immunoprecipitation techniques in conjunction with a nuclease assay. Further analysis of this alkaline deoxyribonuclease in RAD52 during meiosis reveals that its level reaches maximum activity at a time corresponding to the beginning of DNA synthesis and recombination, whereas in a rad52 mutant, the level is low and remains constant throughout. This alkaline deoxyribonuclease has been purified to homogenicity and has been found to be an endo-exonuclease that requires Mg^{2+} for activity and has a molecular weight of about 70 K as determined by SDS-polycrylamide gel electrophoresis.

Principal Investigator and All Other Personnel Engaged on the Project:

T. Chow	Visiting Fellow	CGTB	NIEHS
M. Resnick	Research Geneticist	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Various repair-deficient strains of Saccharomyces cerevisiae are grown using standard techniques. To obtain yeast crude extracts, cells are broken open with a French pressure cell, centrifuged at 4100xg for 20 min and the supernatant examined for nuclease activity. The measurement of the RAD52-controlled 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 mM Tris-HCl, pH8.0, containing 10 mM $MgCl_2$. The purification of the alkaline deoxyribonuclease involves standard column chromatography technqiues. Standard biochemical procedures are used to characterize the deoxyribonuclease.

MAJOR FINDINGS AND PROPOSED COURSE: DNA repair processes are required to protect against external damaging agents and many of the repair mechanisms are involved in mutagenesis and recombination. In Saccharomyces cerevisiae, the RAD52 gene has been genetically identified as controlling DNA repair of ionizing radiation damage and spontaneous and induced recombination. Although, in some cases, the mechanism has been inferred, neither the product of RAD52 nor any other gene products involved in DNA repair in yeast have been identified.

In screening crude extracts of wild-type and repair mutants of yeast, we have found that rad52 strains lack an alkaline deoxynibonuclease that immuno-cross-reacts with an antiserum raised against a Neurospora endo-exonuclease. Furthermore, this alkaline deoxyribonuclease increases in activity at a time corresponding to early stages of meiotic recombination.

This alkaline deoxynibonuclease has been purified to homogenicity and identified to be a protein of molecular weight of 70,000 Daltons. Evidence obtained with a RAD52 clone suggests that this alkaline deoxyribronuclease is not the gene product of RAD52 but the product of an unidentified gene whose expression is controlled by the RAD52 gene. We are currently attempting to characterize and to clone the gene for this alkaline deoxyribonuclease which will allow us to analyze further th biochemical events in recombination and their role in repair.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the genetic events under the control of RAD52 gene in Saccharomyces cerevisiae have been well described, the biochemical events involved are not well understood. The identification of a gene product under RAD52 control, therefore, will allow us to identify the relevant biochemical events involved. Furthermore, this work will allow us to understand the molecular events that surround recombination and DNA-repair and its control, either in mitotically growing cells or meiotically developing systems. The techniques being developed could be used to investigate similar mechanisms in germinal lines of higher organisms.

1. Chow, T., and Resnick, M. A.: The identification of a deoxyribonuclease controlled by the RAD52 gene of Saccharomyces cerevisiae. J. Cell. Biochem., Supp. 7B, pp. 238, 1983.

2. Chow, T., and Resnick, M. A.: The Identification of a Deoxyribonuclease Controlled by the RAD52 Gene of Saccharomyces Cerevisiae. In Friedberg, E. and Bridges, B. (Eds.): Cellular Responses to DNA Damage. Alan R. Liss, Inc., (In press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21020-02 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of recombination induced by DNA damage

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

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:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Associated with G-1 gene conversion events are reciprocal recombination events. These results are consistent with the model proposed by Resnick for the repair of DNA double-strand breaks; biochemical evidence suggests that these breaks are present at the time of cell mating.

Principal Investigator and All Other Personnel Engaged on the Project:
M. Resnick Research Geneticist CGTB NIEHS

PROJECT DESCRIPTION

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. Double-strand breaks induced by ionizing reduction remain unrepaired until the time of mating. 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. We have developed the system further to examine exchange of outside markers, even when cells are in G-1; approximately 30% of the events are associated with outside marker exchange.

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.

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.

PUBLICATIONS

Haber, J., Comeau, A., Liu, P., Rogers, D., Steward, S., Resnick, M.A., and Weiffenbach, B.: Mechanism of Homothallic switching of yeast mating type genes. Recent Adv. Yeast Mol. Biol. 1: 332-347, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21028-02 CGTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Salmonella Plate Test

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Errol Zeiger Supervisory Microbiologist CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of studies are underway which are designed to improve our understanding of the dynamics of the Ames Salmonella/microsome test and to make improvements in the standard protocols to make the test easier to run and/or to make the test results more readily interpretable. These studies include the effects of substances which may be present in environmental mixtures which interfere with a positive mutagenic response in the apparent absence of cellular toxicity; characterization of the new tester strains, TA97 and TA102; the effect of prolonged storage of mutagen solutions used for positive controls; and the kinetics of cell growth on the plate as a function of the number of cells plated, histidine concentration or mutagen concentration.

Principal Investigator and All Other Personnel Engaged on the Project:

E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
D. Pagano	Research Microbiologist	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Salmonella/microsome plate test or preincubation test is being used with occasional modificiation.

MAJOR FINDINGS AND PROPOSED COURSE: Four direct-acting mutagens, sodium azide, 4-nitro-o-phenylenediamine, N-aminomorpholine, and ethylmethanesulfonate were tested in a standard plate test, using Salmonella TA100, as mixtures with varying levels of each of the following compounds: 2-mercaptoethanol, 2-phenylethanol, crystal violet, lauric acid, caprylic acid, deoxycholic acid, tremethoprime, kepone, and butylated hydroxyanisole, which we previously showed could inhibit the Salmonella response. At doses of inhibitor which produced no observable toxicity at 20x magnification of the background lawn, there was a decrease in the number of revertant colonies seen. In most cases, the proportional reduction of his^+ revertants seen in the presence of inhibitor plus mutagen was equivalent to the reduction in the presence of inhibitor alone, implying that the suppressive effects were due to the inhibitor's action on the Salmonella directly and was independent of the mutagen in the mixture. In a few cases the results were mutagen dependent. Changes in individual cell morphology ranging from slight cellular enlargement to filamentation or subtle changes in the microcolonies could be seen at 400x magnification only. Work is continuing on selected mutagen/inhibitor combinations.

Preliminary studies have shown that both new Salmonella tester strains, TA97 and TA102, exhibit a wide range of colony size for spontaneous as well as induced revertants. TA97, additionally, appears to be sensitive to dimethylsulfoxide (DMSO), the standard solvent used for water insoluble chemicals. Both TA97 and TA102 have an increased spontaneous revertant number when plated in the presence of rat liver S9 mix. Studies are continuing on these strains and will be used to determine their usefulness for routine chemical screening.

After 480 days of storage, solutions of 2-aminoanthracene and 4-nitro-o-phenylenediamine have showed a decline in mutagenicity, while 4-nitroquinoline-N-oxide (4-NQO) and benzo(a)pyrene have remained stable. Sodium azide has shown an increase in the revertant number with storage. This study will continue with the inclusion of absorption spectra on each sample in order to compare structural changes with mutagenic changes and the suitability of cold storage for mutagen solutions used as positive controls.

Regardless of the type of medium in which strain TA100 was grown, the cells (when plated at approx. $1x10^8$/plate) show a lag time of about 2 hrs followed by doublings approximately every 45 min to 1 hr until the histidine is exhausted (after approximately 6-8 doublings), after which the cell number remains relatively constant. As expected, decreasing the histidine concentration in the top agar slightly increases the lag time and leads to a lower total number of cell divisions. However, under normal circumstances, the his^+ revertant cells begin to increase in number 6-8 hours after plating, at which point the his^- cells

have already undergone 2-4 divisions. Adding his^+ cells to the top agar, in a reconstruction-type experiment, leads to the same result -- the division of his^+ cells on the plate lags behind his^- cells. Treatment with 4-NQO at a dose which induces >1000 his^+ revertants per plate shortens the his^+ appearance lag by 2 hours, but does not eliminate it completely. Studies are planned to explain this phenomenon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Salmonella (Ames) test is currently the most widely used single assay for detecting chemical mutagens and has an extensive data base to draw upon. Much work has yet to be done to define the dynamics of cell growth and mutagenesis on the plate and the factors that influence these biological phenomena. These studies will provide an improved basis for interpreting data and results from the test and will hopefully lead to protocol modifications which will improve test efficiency and make it easier to run for people with minimal training.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21045-01 CGTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of SPO11, a Gene Required for the Early Events of Meiosis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

C. Giroux Staff Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The spo11 gene is required for the completion of meiosis and sporulation in the yeast, Saccharomyces cerevisiae. The spo11-1 mutant is defective in the early events of meiosis; it fails to undergo meiotic recombination and exhibits extensive aneuploidy following the first reductional chromosome segregation. Unlike other recombination deficient meiotic mutants, however, spo11-1 is not repair deficient and does exhibit normal levels of mitotic recombination. To analyze the function and regulation of this meiosis-specific gene, a genetic system has been devised to allow its physical isolation by cloning following transformation and complementation of the spo11-1 mutations during meiosis. Using this system, a clone bank has been screened and a complementing clone has been isolated. This clone is currently being analyzed by subcloning and genetic mapping in order to verify its identity and to determine the structure of the complementing DNA sequence.

Principal Investigator and All Other Personnel Engaged on the Project:
C. Giroux Staff Fellow CGTB NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Meiosis mutants are grown, sporulated, and recombination frequencies are determined using standard yeast procedures. Cloned DNA sequences are examined for complementation in meiosis by sporulating mutant strains following transformation by, and selection for, the cloned DNA inserted into an E. coli - yeast plasmid shuttle vector. Manipulations of the cloned DNA are performed using standard recombinant DNA procedures.

MAJOR FINDINGS AND PROPOSED COURSE: A yeast DNA sequence which complements the meiosis mutant spo11-1 has been isolated from a total genome clone bank. It will be determined if this clone is the spo11 structural gene by genetic mapping following integration of the recombinant plasmid into the yeast genome. If this clone is the spo11 structural gene, then its gene product and its regulation will be investigated. If this clone is a different gene from spo11, then the properties of this new gene and its interaction with spo11 will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The gene products which mediate meiotic DNA metabolism in germ line cells are at present unidentified. The physical isolation of the spo11 gene would represent the first isolation and identification of a gene product essential for meiosis. This purified gene could then be used as a probe of the mechanism and regulation of meiosis in yeast. This fundamental information is necessary for an understanding of the different genetic susceptibilities and properties of somatic versus germ line cells in response to environmental challenges.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21049-01 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Synthesis and Metabolism During Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

M. A. Resnick Research Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

Dr. Akio Sugino, University of Georgia, Athens, GA

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Unique DNA metabolic activities have been implicated during meiosis and following exposure of mitotic cells to DNA damaging agents. To understand the processes involved, it is important to examine the enzymes that are presumed to be responsible. We are characterizing both the DNA and the DNA metabolic enzymes at various times in meiosis in wild type and repair deficient cells. DNA polymerase I and II increase by approximately two times during meiosis near the time of DNA synthesis and recombination. Single-strand deoxyribonucleases do not exhibit much variation; however, the RAD52 controlled nuclease appears to increase by approximately 3-4 times at about the time of recombination and then decreases at late times of meiosis. It, therefore, appears that there is a coordinated increase in enzyme systems involved in meiotic DNA synthesis and recombination. Based on recent work of Sugino, there appear to be DNA methylation signals for the initiation of DNA synthesis in mitotically growing cells. We are investigating whether such signals also exist in meiotic cells in relation to synthesis and recombination.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

M. A. Resnick	Research Geneticist	CGTB	NIEHS
T. Chow	Visiting Fellow	CGTB	NIEHS
J. Nitiss	Guest Worker	CGTB	NIEHS
J. Westmoreland	Biological Laboratory Technician	CGTB	NIEHS

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. Biochemical analysis of polymerases and nucleases are performed on partially purified crude extracts. Methylated DNA is being examined using restriction enzymes that can discriminate methylated sites. 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 are used as needed.

MAJOR FINDINGS AND PROPOSED COURSE: To understand repair events and the role of various repair genes in meiosis, it is important to characterize the DNA metabolic events that occur during normal meiosis and in various repair deficient mutants. Crude extracts of repair proficient and deficient strains are being examined at various time during meiosis and following exposure to DNA damaging agents for changes in DNA polymerases I and II, single-strand deoxyribonucleases, Mg^{++} dependent and independent nucleases and the specific rad52 controlled deoxyribonucleases. DNA polymerase I increases by nearly a factor of two at the onset of the meiotic round of DNA synthesis while polymerase II increases later at about the time of commitment to recombination. DNA polymerase II appears to be absent in the rad52 mutant when grown under presporulation conditions. The total single-strand deoxyribonucleases that are active at pH8 remain approximately constant throughout meiosis. However, the rad52 controlled nuclease increases by over a factor of three at the time of commitment to recombination, implicating it in the meiotic recombinational process. There is no detectable rad52 nuclease in cells of meiotic rad52 mutants. From these results we have concluded that there is a coordinated increase in enzymes associated with meiotic DNA synthesis and recombination. Observations of 100-1000 fold increases in recombination cannot be ascribed to large increases of these enzymes.

Recent results of Sugino have implicated methylation sites in the process of replication of yeast. He was able to show that at the onset of DNA synthesis origins of replication are methylated and later in the cell cycle they become demethylated. Using his techniques to identify methylated sites with restriction enzymes that discriminate methylated bases, we are beginning to examine DNA of cells undergoing meiosis. There are two goals in this work. The first is to determine whether premeiotic DNA synthesis requires methylation and the second is to examine a possible role for methylation in recombination.

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 enzyme systems 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 21052-01 CGTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Xenobiotics to Mutagens Using Non-hepatic Microsomal Enzyme Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Errol Zeiger Supervisory Microbiologist CGTB NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Pulmonary Function and Toxicology
Laboratory of Developmental and Reproductive Toxicology

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Xenobiotic chemicals can be metabolized by organs and tissues other than the liver. Additionally, the prostaglandin endoperoxide synthetase (PES) system is found in a number of organs and is not dependent on cytochrome P-450. The metabolism of polycyclic aromatic hydrocarbons, aromatic amines, and other chemicals to mutagens in Salmonella tester strains is being studied using PES and, also, microsomal preparations from lungs, testes, and prostate, in addition to liver.

Only the dihydrodiols of polycyclic aromatic hydrocarbons which allow formation of bay region diolepoxides were mutagenic in the presence of PES. The majority of aromatic amines studied were also mutagenic, but a number of them were weak or nonmutagenic for reasons not evident. N-Nitrosamines were not active in this system. Testes and prostate microsomal mixed function oxidases are inducible and homogenates from these organs can metabolize benzo(a)pyrene to a mutagen.

Principal Investigator and All Other Personnel Engaged on the Project:

E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Z. Matijasevic	Visiting Fellow	CGTB	NIEHS
D. Pagano	Research Microbiologist	CGTB	NIEHS
T. Eling	Head, Prostaglandin Group	LFPT	NIEHS
I. Lee		LDRT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: A crude microsomal preparation containing prostaglandin endoperoxide 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. 9000xg homogenates or purified microsomal preparations are made from other organs using standard techniques. The standard Salmonella plate test is used.

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 metabolzed 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.

Of the aromatic amines tested, benzidine, 2-aminofluorene, 2-naphthylamine, and 2,5-diaminoanisole were metabolized to mutagenic products by PES. 1-Naphthylamine, 2-aminoanthracene, 2-acetylaminofluorene, and 2,4-diaminoanisole were negative or weakly mutagenic. Thus, the prostaglandin synthetase system appears more selective than the cytochrome P-450 system in the conversion of polycyclic hydrocarbons and their metabolites and aromatic amines to mutagenic products. The other compounds tested: N-nitrosodimethylamine, N-nitrosomorpholine, the pesticide Aminocarb, and the phthalate derivative di(2-ethylhexyl)phthalate, were not mutagenic.

Studies are underway with different Salmonella strains in order to determine the PES-generated mutagenic metabolic intermediate(s) of 2-aminofluorene.

9000xg homogenates of testes and prostate from rats induced with TCDD and/or a methylating agent were able to metabolize benzo(a)pyrene to a mutagen. Studies are underway with chemicals from different classes.

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 endoperoxide synthetase is ubiquitous throughout mammalian tissues and frequently co-exists with cytochrome P-450. There is increasing evidence that PES may serve as an alternative or complementary activation system in vivo. This work will help to further elucidate the role of PES in the metabolic activation of xenobiotics. In addition, the role of organs other than the liver is being studied to see how their xenobiotic activating capability compares to that of liver.

PUBLICATIONS

Robertson, I.G.C., Sivarajah, K., Eling. T., and Zeiger, E.: Activation of some aromatic amines to mutagenic products by prostaglandin endoperoxide synthetase. Cancer Res. 43: 476-480, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21058-01 CGTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Dosimetry of Ethylmethane Sulfonate in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Z. Matijasevic Visiting Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The mutagenic activity of ethylmethane sulfonate (EMS) as a function of its DNA alkylating ability is being studied in Salmonella typhimurium. The mutagenic activity of EMS in the base-pair substitution strain G-46 and its repair deficient derivatives (TA1950, [uvrB]; TA92, [pKM101]; TA2410,[uvrB, pKM101]) are being compared. The increases in mutation frequencies in these strains will be related to the levels of DNA ethylation and removal. This will provide a reference for the effects of the various repair deficiencies on EMS-induced mutagenesis and will provide data to allow us to relate treated dose to delivered dose to mutagenic response.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

Z. Matijasevic	Visiting Fellow	CGTB	NIEHS
E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mutagenicity determinations are done by suspension assays, except that $1x10^{10}$ cell line are treated, instead of the standard $1\text{-}10x10^{8}$. ^{3}H-EMS will be used in the alkylation studies; total alkylations per nucleotide and the ratios of N-7 to O-6 to other alkylation sites will be determined using standard methods.

MAJOR FINDINGS AND PROPOSED COURSE: This project was recently initiated, and the ^{3}H-EMS is not yet available. Dose-response determinations have been made using the four Salmonella strains, and two types of responses were seen. Strains carrying the uvrB mutation show a threshold effect, whereas strains with wild-type excision repair, with or without the plasmid pKM101, produce a linear response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The estimation of risk for mutagenic agents involves knowing not only the amount of chemical the target cell is exposed to, but the delivered dose as well. That is, the amount of active mutagen that interacts with the target molecule, DNA, to form a premutagenic lesion. This study will provide such data using the standard mutagen, EMS, and the results can be compared with results from similar studies using other bacteria, yeast, fungi, cultured mammalian cells, Drosophila, and mammalian tissues in vivo. This will determine the degree in which mutagenicity results from microbial cells can be extrapolated to mammalian, including human, cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60102-05 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Errol Zeiger Supervisory Microbiologist CGTB NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Reproductive and Developmental Toxicology

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Salmonella strains TA1535, TA1537, TA1538, TA98, TA100, TA97 and TA102 are being used to test chemicals of interest for mutagenicity. Diethylstilbestrol (DES) will be tested for mutagenicity in strain TA102 using various metabolic activation systems. Some antioxidants which have been shown to cause mutagenic suppression in TA100 will be looked at in other strains as well. Pyrene, which has so far been shown to have inconsistent mutagenic results and which is considered an inactive analog of the carcinogenic benzy(a)pyrene, is being studied with the new Ames' tester strain, TA97. Also, the mutagenicity data for ethylnitrosourea is incomplete, so this chemical will be studied using a number of Salmonella tester strains.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
D. Pagano	Research Microbiologist	CGTB	NIEHS
Z. Matijasevic	Visiting Fellow	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test of Ames with some modifications or a liquid suspension test were used.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary studies with strain TA102 have shown that this strain is not compatible with the rat liver S9 mix using the standard protocol. A horseradish peroxidas metabolizing system will be used instead since biochemical studies using this system have shown that it can produce active metabolites of carcinogens. DES is also very insoluble in water, so alternative liquid suspension methods must be found in order to avoid its precipitation. Work will continue in this area.

The antioxidant, sodium bisulfite, has been shown to suppress the mutagenic response of strain TA100 at pH 7.0. When tested in strain TA97 and TA1538, the number of spontaneous revertants was also decreased (at pH 7.0), but at pH 5.2, sodium bisulfite appears to exhibit a mutagenic response in strain TA97. Further work on the effect of pH and the results in other strains will continue.

Preliminary results with pyrene indicate the presence of mutagenic activity on strains TA1537 and TA97. This activity is dependent on the amount of rat liver S9 fraction and is very sharply dose-dependent. Further work using strain TA1137 and TA137 to determine toxicity, as well as mutagenicity studies comparing the plate test with a suspension assay in strain TA97 will be included in the investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Diethylstilbestrol is a known carcinogenic agent to which many people in the population have been exposed. To date the determination of mutagenicity has produced inconsistent results. It would be of great value to determine the mutagenicity (or lack of it) in a strain that is especially sensitive to mutation induction by chemicals similar in structure to some DES metabolites.

The possibility that pH may alter the response of an antioxidant has significance in that this antioxidant is present in foodstuffs at a wide range of pH's. It is of interest to note that the more acidic pH produces the mutagenic results.

Pyrene is found in combustion products as is its relative, benzo(a)pyrene. A positive in vitro mutagenic result in a compound previously considered non-mutagenic in in vitro tests but positive in other in vitro assays may provide some important structure activity information for comparing possible metabolic activation mechanisms.

Ethylnitrosourea is widely used in mammalian germ cell mutation assays because of its strong mutagenicity. However, its mutagenicity has been poorly characterized in microbial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60105-05 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Control of Mutation in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. M. Mason Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

B. Slatko, Dept. of Biology, Williams College, Williamstown, MA
E. Strobel, Dept. of Biological Sciences, Purdue University, West Lafayette, IN
M. M. Green, Dept. of Genetics, University of California, Davis, CA

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mutation rates are 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. This mutator blocks repair of chromosome breaks specifically in oocytes allowing a previously undescribed repair process to be observed. In this process, broken chromosomes are "healed", allowing the recovery of terminal deletions. (2) The interaction of DNA repair-defective mutants and transposable elements has been observed in double mutant combinations. None of the repair-defective mutants used influenced the rates of transposon-induced mutation or recombination, although mutants at the mei-41 locus prevented the transmission of transposon-bearing chromosomes.

Principal Investigator and All Other Personnel Engaged on the Project:

J. M. Mason	Geneticist	CGTB	NIEHS
L. Champion	Biological Laboratory Technician	CGTB	NIEHS

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 do not appear to be capped by a previously existing telomere. The mutator is recessive and maps near the end of the left arm of chromosome III. It is active throughout oocyte development, but not during spermiogenesis. The mutator does not increase the frequency of meiotic recombination or nondisjunction. It does, however, increase the frequency of X-chromosome loss. These losses are the result of whole arm deletions in which the centromere is recovered but the rest of the X has been lost. The termini of a number of terminally deleted chromosomes are being cloned in order to sequence them and develop a consensus sequence for functional telomeres in Drosophila.

Transposable elements in Drosophila cause a wide range of genetic effects including mutation, mitotic recombination, chromosome aberrations and sterility. These effects have been termed "hybrid dysgenesis". The control of transposition is very poorly understood in any system. We have asked whether cellular functions are important in the control of transposition by combining mutants defective in DNA repair and recombination with transposable elements. If such functions are important for transposition, the transposon-induced frequencies of mutation and recombination should be altered. They are not. Mutants at four different loci had no effect on either mutation or recombination. Mutants at the mei-41 locus, however, drastically reduced the recovery of transposon-bearing chromosomes in the following generation. The mechanism for this is unknown, but loss of transposon-bearing chromosomes is correlated with dominant lethality.

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

Slatko, B. E., Mason, J. M., and Woodruff, R. C.: Transposable mutator elements in Drosophila melanogaster can function despite nonfunctional host DNA repair enzymes. Genetical Research (in press) 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60106-05 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. M. Mason Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

J. E. Boyd, Dept. of Genetics, University of California, Davis, CA
E. Strobel, Dept. of Biological Sciences, Purdue University, West Lafayette, IN

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project

J. M. Mason	Geneticist	CGTB	NIEHS
L. Champion	Biological Laboratory Technician	CGTB	NIEHS

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) 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. (c) 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 150 kb. To confirm that the mei-41 locus is large at the DNA level and to gain some insight into the structure and control of a large locus, new mei-41 mutations will be made using transposons and the locus will be cloned.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 60122-04 CGTB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of DNA Repair in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

M. Resnick Research Geneticist 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 ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. 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. This is being tested using sporulation mutants that do not undergo reduction/division; the meiotic products are, therefore, diploid.

Principal Investigator and All Other Personnel Engaged on the Project:

M. Resnick	Research Geneticist	CGTB	NIEHS
J. Westmoreland	Biological Lab Technician	CGTB	NIEHS

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 throughout meiosis. 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. To test whether lethality is due to nondisjunction we are using sporulation mutants that bypass meiosis I (reductional division) and thereby give use to diploid spores.

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 recombinational 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, MA., Game, J.C., and Stasiewicz, S.: The genetic effects of UV on excision proficient and deficient yeast during meiosis. Genetics (In Press, 1983).

Resnick, M.S., Stasiewicz, S., and Game, J.C.: Meiotic DNA metabolism in wild type and excision-deficient yeast following UV exposure. Genetics (In Press, 1983).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60123-04 CGTB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Repair Processes During Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

M. Resnick Research Geneticist 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:	PROFESSIONAL:	OTHER:
1.9	0.9	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using the yeast Saccharomyces cerevisiae we are examining mitotically identified DNA repair systems during 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 pre-meiotic DNA synthesis and their occurrence coincides with commitment to cell death. Since these SSI's serve as a primary site in in vitro DNA synthesis assays, it is possible to isolate and characterize them. 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, although the mechanism of death differs in the two mutants.

Principal Investigator and All Other Personnel Engaged on the Project:

M. Resnick	Research Geneticist	CGTB	NIEHS
T. Chow	Visiting Fellow	CGTB	NIEHS
J. Westmoreland	Biological Lab Technician	CGTB	NIEHS
J. Nitiss	Guest Worker	CGTB	NIEHS

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. DNA is gently isolated for further molecular characterization.

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 synthesis; 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. Using cloned probes and hybridization techniques, we are attempting to identify individual chromosome peaks in sucrose gradients, and, therefore, greatly enhance the sensitivity of the assay. 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 that 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

the appearance of the DNA interruptions and indicates that unresolved recombination structures may cause lethality. Procedures have been developed for the gentle isolation and restriction of large molecular weight DNA so as to recover pieces of DNA containing SSI's. The nature of the SSI's is being evaluated using in vitro synthesis at the site of SSI's and isolation and characterization of the newly synthesized DNA.

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. In the absence of the RAD50 gene product, no chromosome interactions occur and based on the kinetics of cell death, cells die late in meiosis because of problems of chromosome segregation. 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. To improve our levels of discrimination, we are attempting to synchronize the meiotic events using cell elutriation procedures.

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

Resnick, M.A., Nitiss, J., and Game, J.C.: The role of repair genes during meiosis in yeast. J. Cellular Biochemistry 7B: 237, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60128-03 CGTB

PERIOD COVERED: October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Collaborative Study to Test for "Genetic Drift" in Laboratory Stocks of Ames' Strs.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation): E. Zeiger Supervisory Microbiologist 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:
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of a 38-laboratory international study individual laboratory stocks of five Salmonella typhimurium tester strains were compared with reference strains in their response to a mutagen, 4-nitroquinoline-N-oxide. The results from all laboratories were analyzed in order to determine the levels of agreement within and between laboratories for each Salmonella strain. It was concluded that "genetic drift" was not a significant factor in interlaboratory variability.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

E. Zeiger	Supervisory Microbiologist	CGTB	NIEHS
M. Shelby	Geneticist	CGTB	NIEHS
B. Margolin	Mathematical Statistician	BRAP	NIEHS
K. Risko	Mathematical Statistician	BRAP	NIEHS

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 Salmonella plate test protocol. Strains were also checked for known characteristics.

MAJOR FINDINGS: The data from the various laboratories were analyzed and the responses compared. Evaluations showed that the majority of laboratories do not show significant differences in control values between the inhouse and reference cultures. Also, the variances around the control means of the two cultures are not significantly different (with the exception of occasional outliers). Two European laboratories also analyzed 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 interlaboratory variation and will provide information as to what portion of the variation seen is a function of the Salmonella culture used, or other laboratory related factors. This information is needed in order to adequately evaluate data from NTP and other testing laboratories.

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: $330,000

PROJECT DESCRIPTION

OBJECTIVES: The major specific aim has been to derive experimental approaches and molecular probes for detection of DNA sequence transposition in mammalian cells upon carcinogenic insults. The endogenous (germ-line inherited) proviral genome of RFM/Un strain mice has served as the experimental model. The present studies have focused on molecular approaches to provide evidence for retrovirus related gene rearrangements in radiation induced hematopoietic neoplasias.

METHODS EMPLOYED: Normal and neoplastic RFM strain cells were cultivated in vitro, with particular emphasis on hematopoietic tissues. Endogenous retroviruses were induced by chemical treatment of cultured cells and characterized biologically and biochemically. These viruses have been used to prepare total genomic probes and have also been molecularly cloned for detailed analysis by restriction endonuclease mapping and nucleotide sequencing techniques. The cloned viral genomes have also been used as precisely defined total and subgenomic region probes. Male RFM/Un mice were x-irradiated to induce myelogenous leukemia and other hematopoietic neoplasias. Restriction endonuclease mapping and Southern gel blotting were used to characterize the proviral DNA structure in normal and primary neoplastic tissues and an established radiogenic myelogenous (Upton) cell line.

MAJOR FINDINGS AND PROPOSED COURSE: Major findings of this reporting period include: 1) the established radiogenic myelogenous leukemia (Upton) cell line was shown to contain additional copies of the endogenous ecotropic RFV proviral DNA at new integration sites; 2) of various hematopoietic primary neoplastic tissues examined, myelogenous leukemia tissues (of x-ray irradiated RFM/Un mice) and also reticulum cell sarcoma (mostly in non-irradiated aged RFM/Un mice) exhibited additional copies of the endogenous ecotropic RFV provirus at new integration sites. The germ line provirus located on chromosome 5 is apparently unchanged; 3) no additional RFV proviral DNA copies were evident in radiation induced thymic lymphoma of RFM/Un mice; and 4) preliminary evidence indicates that the RFM restriction of endogenous ecotropic virus is not linked to the mouse Fv-1 gene, but similarly involves the viral gag gene product as the target.

Ongoing and future studies will include: 1) genetic crosses of RFM/Un mice with BALB/c mice to investigate the segregation of the genetic locus responsible for the RFM restriction of endogenous ecotropic virus; 2) studies on oncogene

expression in primary myelogenous leukemic and other neoplastic tissues; and 3) isolation of somatic cell hybrids between hamster and both established radiogenic myelogenous leukemic cell lines and primary myelogenous leukemic cells for the purpose of chromosome localization of the additional ecotropic provirus copies.

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

DATES AGREEMENT INITIATED: 1. September 22, 1978 (222-YO1-ES-80038)
2. April 1, 1981 (222-YO1-ES-10063)

CURRENT ANNUAL LEVEL: $595,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this interagency agreement with the Department of Energy are to identify the mutagens produced in foods, primarily beef products, 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 boiled 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. Standard separation chemistry procedures are being used to isolate, purify and identify individual mutagenic components of these extracts.

MAJOR FINDINGS AND PROPOSED COURSE: Hamburger: a series of extraction procedures were developed which greatly increased 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 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 been confirmed. Cold IQ and radiolabelled IQ have been synthesiz(Me-IQ will be synthesized in the near future. A number of mutagen-containing chromatographic peaks have been isolated and purified and the mutagens present are being subjected to high resolution mass spectrum analysis.

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 metabolites 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. Adduct formation by IQ and Trp-P-2 is being measured and compared in Salmonella and CHO cells in an attempt to determine the reason for the virtual absence of IQ mutagenicity in CHO cells.

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 than 500 MW in the soluble portion of the beef extract. $FeSO_4$ addition further stimulates 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 coelutes with IQ and one which may be identical to Trp-P-2.

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 to 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 future 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. Bjeldanes, L.F., Morris, M.M., Felton, J.S., Healy, S., Stuermer, D., Berry, P., Timourian, H., and Hatch, F.T.: Mutagens from the cooking of food. II. Survey of mutagen formation in the major protein-rich foods of the North American diet. J. Food and Chem. Toxicol. 20: 357-363, 1982.

2. Bjeldanes, L. F., Morris, M.M., Felton, J.S., Healy, S., Stuermer, D., Berry, P., Timourian, H., and Hatch, F.T.: Mutagens from the cooking of food. III. Secondary sources of cooked dietary protein. J. Food and Chem. Toxicol. 20: 365-369, 1982.

3. Hatch, F.T., Felton, J.S., and Bjeldanes, L.F.: Formation of Mutagens in Protein Foods During Traditional Cooking Procedures. In Stich, H., and Powrie, W. (Eds.) Carcinogens and Mutagens in Food, Critical Reviews of Toxicology (In press)

4. Grose, K.D., Bjeldanes, L.F., Stuermer, D.H., Davis, P., Healy, S.K., and Felton, J.S.: An XAD-2 resin method for extraction of mutagens from fried ground beef. Mutat. Res. Lett. 105: 43-49, 1982.

5. Bjeldanes, L.F., Morris, M., Timourian, H., and Hatch, F.T.: Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. J. Agric. and Food Chem. (In Press)

6. Grose, K.R., Kim, I., and Bjeldanes, L.F. Deuteration of mutagenic aromatic nitrogen heterocycles derived from protein and amino acid pyrolysates. J. Agric. and Food Chem. 30: 766-768, 1982.

7. Hatch, F.T., Felton, J.S., Thompson, L.H., Carrano, A.V., Healy, S.K., Salazar, E.P., and Minkler, J.L.: Comparative genotoxic effects of the cooked food related mutagens Trp-P-2 and IQ in bacteria and cultured mammalian cells. Mutat. Res. (In Press)

8. Taylor, R.T., Shore, V., and Fultz, E. Mutagen formation in a model beef boiling system: Stimulation studies with amino acids and other agents. Environ. Mutag. 4: 368, 1982.

9. Moore, D., and Felton, J.S.: A microcomputer program for analyzing Ames test data. Mutat. Res. Lett. 119: 95-102, 1983.

10. Felton, J.S., Healy, S.K., and Hatch, F.T. Mutagenic activation of cooked ground beef by human liver microsomes. (Submitted).

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 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: The dose response curve in ENU treated spermatogonia has been completed down to 50 mg/kg and has confirmed the curve as nonlinear. Acquisition of additional data at 25 mg/kg will depend on results from analysis of existing data. The dose fractionation experiments have also been completed using 10 mg/kg ENU each week for ten weeks. The induced mutation rate is only 12% of that obtained with a single 100 mg/kg exposure indicating the efficient repair of ENU induced damage in the DNA. In germ cell stage sensitivity studies using 50 mg/kg ENU, evidence has been obtained indicating a sensitive stage observed 30-40 days post treatment. This period corresponds to differentiating spermatogonia or early spermatocytes and is the cell stage also sensitive to N-methyl-N-nitrosourea. Experiments in ENU treated females have shown no evidence of induced mutations in mature or maturing oocytes. Molecular dosimetry studies have been extended down to 2.5 mg/kg and a linear relationship between administered dose and ethylation of testicular DNA demonstrated. Preliminary dosimetry experiments in females indicate a higher level of DNA ethylation in the ovaries than in the testes. Both dibromochloropropane and hexamethylphosphoramide are on test in the specific locus assay. No evidence for germ cell mutagenicity has been obtained to date. Urethane is scheduled for testing, and two additional chemicals will be identified for testing in the coming year.

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.

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

CURRENT ANNUAL LEVEL: $101,106

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 were developed that allow Drosophila to be used to test for chemically induced aneuploidy. The questions that were addressed during this portion of the project were: (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: The above issues have been settled. (1) Low level effects are best scored by monitoring segregation of attached-X from Y (both gain and loss). 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 for the presence of triploids. These three 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 chemical will be tested at two concentrations using a sample size of 10,000 per concentration. This will allow the detection of an induced frequency of 10^{-3} chromosome gains with a power of 0.7 or an induced frequency of 2×10^{-3} gains with a power of 0.95. Large sample sizes can easily be screened for two reasons: (1) a single generation test is used and (2) flies can be weighed instead of counted. This procedure has been shown to be accurate to within 1%.

A protocol has been written based on these findings, and testing has begun on coded control chemicals. The first chemicals tested included: mono-functional alkylating agents, long chain fatty acids, and inhibitors of energy metabolism. Preliminary tests indicate the system is sensitive to these agents.

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 that 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 developed test to detect such chemicals on a large scale.

1. UNIVERSITY OF CALIFORNIA - Berkeley, California
(NO1-ES-1-5004)

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

CURRENT ANNUAL LEVEL: 1. $120,992
2. $18,400

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.

METHODS EMPLOYED: In the development of a meiotic aneuploidy test system, the contractors are devising a means for following chromosome gain among the haploid products of meiosis. The system relies on screening for differences in gene dosage at the CUP1 and ARG4 loci. For the case of aneuploidy among mitotically growing cells, a system is being 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 recombinational events. The mitotic system is a refinement of a previously published system in that it enables a clearer discrimination against false positives. Some agents have been found to act only in combination with cold shock. Conditions are being developed for reproducibly identifying true aneuploids 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 some steps in tumor promotion. A few chemicals are known to induce only aneuploidy 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) that cause changes in chromosomal number in addition to being mutagenic.

PUBLICATIONS

Zimmermann, F.D.: Mutagenicity screening with fungal systems. Ann. N. Y. Acad. Sci. (In Press)

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

CURRENT ANNUAL LEVEL: $72,001

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 has investigated modifications of the Salmonella preincubation protocol with liver S-9 which permit reductive metabolism followed by oxidative metabolism to measure the mutagenicity of benzidine-containing dyes. In addition, rat cecal flora preparations were used to develop an alternate activation system which is representative of the metabolism that occurs in the gut.

MAJOR FINDINGS AND PROPOSED COURSE: The majority of effort on this contract 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, dichlorobenzidine and some model benzidine-based dyes have been evaluated. Also, the sensitivity of detection of benzidine metabolites in rat urine has been determined in preparation for a survey of urines from rats administered benzidine dyes. A number of benzidine-congenor dyes have been tested for mutagenicity using the rat cecal flora metabolic system and, with the exception of one dye, have all been mutagenic. Efforts are underway to relate the levels of mutagenicity to that expected from an equimolar concentration of benzidine congenor. As an extension of this effort, chemicals other than benzidine dyes suspected of being activated via a reductive step will also be tested in the rat cecal flora and liver reductive systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The standard Salmonella 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 initially, or 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.

PUBLICATIONS

Reid, T.M., Morton, D.C., Wang, C.Y., and King, C.M.: Conversion of Congo red and 2-azoxyfluorene to mutagens following in vitro reduction by whole-cell rat cecal bacteria. Environ. Mutag. (In Press).

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

CURRENT ANNUAL LEVEL: 1. $202,840
2. $110,446

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, and aneuploidy have been investigated. Once an acceptable protocol is developed, a number of coded chemicals will be tested.

METHODS EMPLOYED: Allied Corporation and Bioassay are using a Chinese Hamster Ovary (CHO) line to standardize culture and treatment conditions for studying sister chromatid exchange, mutation frequency and chromosomal aberrations. Allied has also done preliminary mutagenic and cytogenetic studies using a human cell line. Studies have also been done on unscheduled DNA synthesis and aneuploidy in the CHO cells.

MAJOR FINDINGS AND PROPOSED COURSE: The majority of effort in these contracts 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 few standard mutagens. Several endpoints have been examined in CHO cells and the human epithelial cell line, HSBP. A protocol has been finalized to study sister chromatid exchange, mutation frequency and chromosomal aberrations in synchronized CHO cells. Each contractor will test 5 coded chemicals to validate the protocol. Once the protocol is validated the laboratories will begin the testing of coded chemicals in CHO cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The major 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 are 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 vivo*, but one wants to estimate the sum of both effects.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC.
Lexington, Kentucky 40503
(NO1-ES-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: $177,530

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to test 30 coded chemicals per year 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 three 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 objective of the project was met with the successful testing of 30 coded chemicals for the induction of SCE and chromosome aberrations.

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.

ARGONNE NATIONAL LABORATORY
Argonne, Illinois 60439
(Y01-ES-20102)

ARTHUR D. LITTLE, INC.
Cambridge, Mass.02140
(N01-ES-15794)

MICROBIOLOGICAL ASSOCIATES
Bethesda, Maryland 20016
(N01-ES-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 (Y01-ES-20102)
Dr. A. Tu (N01-ES-15794)
Dr. R. Kouri (N01-ES-15758)

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: Y01-ES-20102 = $317,915
N01-ES-15794 = $278,604
N01-ES-15758 = $221,969

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 included: 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. 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. The goals for the second year are: 1) preparation

of a publication by the three labs detailing the results of work on model chemicals; 2) establishment of working criteria for acceptability and evaluation of the assay; 3) having the three laboratories perform preliminary toxicity and transformation assays on a group of selected coded compounds; 4) comparison of the results to determine the degree of interlaboratory reproducibility; and 5) resolve any technical differences. Progress has been seen in all these areas and work is continuing. When the stated goals are realized, the laboratories will begin testing other selected 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-ES-15795)

NORTHROP SERVICES, INC.
Research Triangle Park, NC 27709
(NO1-ES-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-ES-15795)
Dr. George Hatch (NO1-ES-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: September 30, 1981

CURRENT ANNUAL LEVEL: NO1-ES-15795 = $272,229
NO1-ES-15796 = $234,172

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 included: 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. These goals are being met and technical aspects are being clarified. The protocol has been slightly modified to optimize the assays response to chemical treatment and working criteria for acceptability and evaluation of the assay have been adopted.

The goals of the second year are: 1) preparation of a joint publication detailing the results of work on model chemicals; 2) having the two laboratories perform toxicity and transformation assays on a group of coded compounds; 3) comparison of the results to determine the degree of interlaboratory reproducibility; 4) resolve any technical differences and establish standard criteria for acceptability and evaluation; and 5) establish a standard protocol. Progress has been seen in all these areas and work is continuing. When the above goals are realized, the two laboratories will begin testing coded compounds.

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.

BIOTECH RESEARCH LABS
Rockville, Maryland 20014
(NO1-ES-15807)

LITTON BIONETICS
Kensington, MD 20895
(NO1-ES-15797)

NORTHROP SERVICES, INC.
Research Triangle Park, NC 27709
(NO1-ES-15798)

TITLE: Task III - Mammalian Cell Transformation Retrovirus Infected Rat Cells

CONTRACTORS' PRINCIPAL INVESTIGATORS: Dr. R. Ting (NO1-ES-15807)
Dr. J. Poiley (NO1-ES-15797)
Dr. W. Suk (NO1-ES-15798)

PROJECT OFFICER (NIEHS): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: NO1-ES-15807 = $127,741
NO1-ES-15797 = $393,815
NO1-ES-15798 = $262,363

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 a modified aggregation (survival) assay which detects the preferential ability of transformed cells to survive under the test conditions.

MAJOR FINDINGS AND PROPOSED COURSE: The major goals for the first year of this study were: 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). These goals have or are being met, although some technical aspects require further evaluation. The protocol has been modified to optimize the assays' response to chemical treatment and working criteria for acceptability and evaluation of the assay have adopted. The goals of the second year are: 1) preparation of a joint publication

detailing the current results (using model compounds); 2) having the three laboratories perform preliminary toxicity and transformation assays on a group of selected coded chemicals; 3) comparison of the results to determine the degree of interlaboratory reproducibility; and 4) resolve technical differences.

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.

ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, NC 27711
(YO1-ES-20079)

TITLE: CGT Rapid Test Response

WORK PERFORMED AT THE FOLLOWING CONTRACT LABORATORIES:
SRI, International (Dr. David Jones, PI)
Litton Bionetics, Inc. (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: November 29, 1982

CURRENT ANNUAL LEVEL: $563,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to test 30-40 chemicals for their potential genetic toxicity in two test components which are part of a complimentary group of short-term tests which were selected to provide multiple test systems and endpoints. These two test components: 1) the in vivo - in vitro (host activated) rat hepatocyte DNA repair assay; and 2) the in vitro transformation of Balb/c 3T3 cell assay, measure direct DNA damage/repair and altered gene expression respectively. The results from these tests will contribute substantially to a data base which will permit an evaluation of the genetic toxicity potential of the chemicals tested.

METHODS EMPLOYED: The chemicals selected will be coded and, when possible, they will be taken from the same chemical lot that has been prepared for the bioassay. The chemicals will be tested according to established protocols in: 1) the in vivo - in vitro (host activated) rat hepatocyte DNA repair assay; and 2) the in vitro transformation of Balb/c 3T3 cell assay. Where appropriate, the protocol has been designed to provide dose-response data.

MAJOR FINDINGS AND PROPOSED COURSE: The chemicals selected for test will include the 1983 bioassay-candidates as well as chemicals selected for retrospective evaluation. Another group of chemicals will be selected from those now currently under test in the chronic bioassay. The expiration date of this agreement is November 30, 1983.

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 two 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.

ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, NC 27711
(YO1-ES-20079)

TITLE: CGT Rapid Test Response - Component II

WORK 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

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project was to test the 19 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 were 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 were 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 were taken from the same chemical lot that had been prepared for the bioassay. Where appropriate, protocols were designed to provide dose-response data.

MAJOR FINDINGS AND PROPOSED COURSE: This contract was terminated November 30, 1982. The 19 1982 bioassay-candidate chemcials were tested in each of the five test components. In addition, the testing of three to eight other chemicals of special interst to NTP was completed in the same test components. The results of these tests are presently undergoing evaluation. A data management system has been developed which provides a capability for obtaining tracking and summary files on the chemicals assigned for genetic toxicity testing in the multi-component testing systems.

New contracts have been negotiated for: 1) the in vitro transformation Balb/c 3T3 cell assay; and 2) the in vivo - in vitro (host activated) rat liver hepatocyte DNA damage/repair assay.

The testing capability for the remaining three test components will be realized through other CGTB/NTP contracts.

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.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(Y01-ES-20085)

TITLE: Heritable Translocation Tests in Mice

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. W. M. Generoso

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE INITIATED: June 1, 1982

CURRENT ANNUAL LEVEL: $152,220

PROJECT DESCRIPTION

OBJECTIVES: This project is being conducted to obtain test data on the capacity of six chemicals to induce heritable chromosomal damage in mammalian germ cells. Test chemicals will be selected from those being tested in mouse specific locus assays.

METHODS EMPLOYED: Routes of administration are determined individually for each chemical. Preliminary studies will include both toxicity and dominant lethal tests. Tests are set up to produce 1000 control and 500 test group male progeny. Sterility or semisterility in F_1 males is determined by the sequential breeding procedure. All sterile or semisterile F_1 males are examined cytologically to confirm the presence of a translocation.

MAJOR FINDINGS AND PROPOSED COURSE: A preliminary study of animals exposed to ethylene oxide by inhalation has shown a very high frequency of translocation carriers. More extensive studies are being conducted at three exposure levels, but no results are yet available on these groups. Dominant lethal studies have been carried out with dibromochloropropane (DBCP) with no effect on fertility or dominant lethal frequency by either intraperitoneal or subcutaneous injection. Dominant lethal studies in treated females are planned. Hexamethylphosphoramide has also given negative results in dominant lethal studies contrary to results reported in the literature. Testing is planned for both urethane and tetrahydrocannabinol.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Translocations represent one of the three major categories (gene mutations, chromosomal damage, aneuploidy) of genetic events known to be associated with human genetic disease. The mouse heritable translocation test (HTT) is currently the only practicable method for detecting and quantifying induced heritable chromosomal damage in mammals. Results from the HTT provide a qualitative assessment of a chemical's potential for inducing translocations as well as quantitative data for use in genetic risk assessment efforts.

BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973
(Y01-ES-20098)

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: $173,287

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 the protocol has been tested and accepted, approximately 20 chemicals per year will be tested for in vivo mutagenicity.

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: Following administration of either mitomycin C cyclophosphamide or 7,12-dimethylbenzanthracene, the frequency of chromosomal aberrations is the same in cells obtained from animals with or without bromodeoxyuridine. These results support the use of BU treated animals for both SCE and aberration scoring. The scoring of BU substituted cells has the added advantage of allowing the identification of first division cells for aberration work. Studies on SCE frequencies when the test chemical is administered at various times relative to BU tablet implantation have confirmed that, for routine testing, test agents can be administered shortly following BU implantation.

A protocol for testing has been tentatively agreed upon and is being used to test five coded chemicals (four chemicals common to both laboratories). Once the data have been analyzed and compared and any necessary adjustments made in the protocol, testing of selected NTP chemicals will begin.

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.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(Y01- ES-20099)

TITLE: Chromosome Aberrations and Sister-Chromatid Exchanges in Human Lymphocytes

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Michael Bender

PROJECT OFFICER (NIEHS): Michael D. Shelby, Ph.D., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $160,752

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 in 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: Frequencies of sister-chromatid exchanges and chromosomal aberrations have been determined in approximately 100 subjects and includes serial sampling in several subjects. The average frequencies so far determined are: SCE = 8.7 ± 1.3 s.d.; chromatid deletions = 0.77 ± 0.07 s.d.; all chromatid aberrations = 1.05 ± 0.08 s.d.; rings and dicentrics = 0.12 ± 0.03 s.d.

A questionnaire and a stratified random sampling scheme have been devised for selection of an additional 300 subjects. A computer data file has been developed for the storage and analysis of all cytogenetics data from this project. Scoring of approximately 150 samples per year is planned in the last two years of the project.

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 frequencies of chromosome aberrations and SCEs and the sources of vairability will, along with standardized protocols with demonstrated interlaboratory reproducibility, permit better design and interpretation of future human cytogenetic monitoring and surveillance studies.

OAK RIDGE ASSOCIATED UNIVERSITIES
Oak Ridge, Tennessee 37830
(Y01-ES-20100)

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: $175,228

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 the protocol has been tested and accepted, approximately 20 chemicals per year will be tested for in vivo mutagenicity.

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: Following administration of either mitomycin C cyclophosphamide or 7,12-dimethylbenzanthracene, the frequency of chromosomal aberrations is the same in cells obtained from animals with or without bromodeoxyuridine. These results support the use of BU treated animals for both SCE and aberration scoring. The scoring of BU substituted cells has the added advantage of allowing the identification of first division cells for aberration work. Studies on SCE frequencies when the test chemical is administered at various times relative to BU tablet implantation have confirmed that, for routine testing, test agents can be administered shortly following BU implantation.

A protocol for testing has been tentatively agreed upon and is being used to test five coded chemicals (four chemicals common to both laboratories). Once the data have been analyzed and compared and any necessary adjustments made in the protocol, testing of selected NTP chemicals will begin.

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.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(Y01-ES-20101)

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., Head, Mammalian Mutagenesis Section, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $107,050

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 in 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: Frequencies of sister-chromatid exchanges and chromosomal aberrations have been determined in approximately 100 subjects and includes serial sampling in several subjects. The average frequencies so far determined are: SCE = 8.7 ± 1.3 s.d.; chromatid deletions = 0.77 ± 0.07 s.d.; all chromatid aberrations = 1.05 ± 0.08 s.d.; rings and dicentrics = 0.12 ± 0.03 s.d.

A questionnaire and a stratified random sampling scheme have been devised for selection of an additional 300 subjects. A computer data file has been developed for the storage and analysis of all cytogenetics data from this project. Scoring of approximately 150 samples per year is planned in the last two years of the project.

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 frequencies of chromosome aberrations and SCEs and the sources of vairability will, along with standardized protocols with demonstrated interlaboratory reproducibility, permit better design and interpretation of future human cytogenetic monitoring and surveillance studies.

RESEARCH TRIANGLE INSTITUTE
Research Triangle Park, North Carolina 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: $423,430

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 three environmentally significant chemicals for germ cell mutagenicity.

METHODS EMPLOYED: Induced mutant frequencies are determine by treating one parent (C57B16J 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 form 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: Data continue to accumulate in experiments to define the ENU dose response curve in spermatogonia. Preliminary evidence of an ENU effect in the presterile breeding period of males has been obtained as has evidence of an effect in treated females. It is possible that an increased mutant frequency may occur among progeny conceived in the first week following female treatment.

A pre-existing beta-thalassemic mutant mouse was discovered in the course of routine electrophoretic screening. Heritability has been confirmed and mutant animals provided to other laboratories for further research.

Analysis of progeny from parents exposed to 200 ppm ethylene oxide is underway. Among 850 progeny conceived in the first week following exposure, three morphological variants but no electrophoretic variants have been observed. The only genetically confirmed variant to date is a coat color mutant. Analysis of the F_1 is ongoing. At present a second inhalation test is underway with DBA males being exposed to 20 and 50 ppm ethylene dibromide.

Work will continue with ENU on the dose response curve, cell-stage sensitivity and effects in females. Breeding of ethylene dibromide exposed mice will begin after six months exposure. Based on the outcome of the ethylene oxide experiment, either additional inhalation studies will be conducted or a third compound will be chosen for testing.

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.

1. LITTON BIONETICS, INC. - Kensington, Maryland
(NO1-ES-28036)

2. INVERESK RESEARCH INTERNATIONAL, LTD. - Musselburgh, Scotland
(NO1-ES-28037)

TITLE: Mouse Lymphoma Bioassay

CONTRACTOR'S PROJECT DIRECTORS: 1. Brian Myhr, Ph.D. (NO1-ES-28036)
2. Douglas McGregor, Ph.D. (NO1-ES-28037)

PROJECT OFFICER (NIEHS): William J. Caspary, Ph.D.

DATE CONTRACTS NEGOTIATED: September 30, 1982

CURRENT ANNUAL LEVEL: 1. $458,005.
2. $272,364

PROJECT DESCRIPTION

OBJECTIVES: The objective of this study is to assess the mutagenic activity of chemicals using the forward mutation assay in L5178Y mouse lymphoma cells. A secondary objective is to optimize the exogenous, metabolic activation system used in these studies.

METHODS EMPLOYED: The major emphasis during the initial phase of the study consisted of optimizing the recipe for the exogenous, metabolic activation system (S-9 preparation). The S-9 mix consisted of the cofactor solution and the liver preparation (S-9). The S-9 consisted of homogenized liver from aroclor 1254 induced Fischer rats which have been centrifuged at 9000 g for 10 minutes. The S-9 was prepared separately by the two laboratories using a common source of Fischer rats. The cofactor solution consisted of an NADPH generating system which was composed of NADP and isocitrate or NADP and glucose-6-phosphate (G-6-P) or NADP, G-6-P, magnesium chloride and potassium chloride.

By monitoring the NADPH concentration at 340 nm, the ratio of NADP to G-6-P which maximizes the rate and amount of NADPH formation was determined. A similar experiment, in which the ratio of NADP to isocitrate or G-6-P was varied, but which used mutation frequency as an end point, was performed to determine if the optimum ratio of components had any detrimental biological consequences. Then, the relative amounts of S-9 and cofactor solution necessary to achieve the optimal induction of mutants for different test chemicals representing different chemical classes was determined. Upon obtaining a satisfactory composition for S-9 mix, the absolute amount of S-9 mix needed per culture to induce optimal mutant frequencies was determined.

MAJOR FINDINGS AND PROPOSED COURSE: Spectrophotometric titrations of isocitrate against a constant concentration of NADP indicated that a 5 to 10:1 molar ratio of isocitrate to NADP or a 2 to 5:1 molar ratio of G-6P to NADP would insure complete conversion of NADP to NADPH and also allow the recycling of NADPH used during the metabolic conversion of the test chemical. At lower molar ratios with G-6-P, the NADPH concentration increases to a maximum then decreases, presumably due to a reaction between NADPH and an endogenous compound.

Treatment with 2-acetylaminofluorene (2-AAF) and 3-methylcholanthrene (3-MCA) was used to assess the effects of increasing amounts of S-9 added to a constant amount of isocitrate containing cofactor solution on the mutant frequency and toxicity. The S-9 concentration in the cultures was increased from 0 to 100 ul/ml for each of the three NADP solution concentrations yielding a final concentration of 0.5 mM, 1 mM and 2 mM NADP. The isocitrate to NADP ratio was 10:1. The results for 3-MCA suggested that a maximal response occurred near 1mM NADP for S-9 concentrations of 50 - 60 ul/ml, although S-9 from 20 to 70 ul/ml was nearly as good. Parallel experiments using 5:1 G-6-P:NADP for the cofactor solution gave similar results, both with respcet to mutagenic response and toxicity. For 2-AAF, however, the response tended to increase with increasing NADP concentration, but 1 mM NADP was certainly adequate in combination with S-9 concentrations ranging from 50 ul/ml to 100 ul/ml. Again, a cofactor solution containing G-6-P gave similar results.

To determine the effects of increasing amounts of S-9 mix on the mutagenic activities of 2-AAF, 3-MCA and DMN, an S-9 formultation of 10 ul/ml S-9, 0.15 mM NADP and 0.75 mM isocitrate or G-6-P was used. The S-9 mix was varied from 0 to an amount that would result in 100 ul/ml of S-9 in the cell cultures. The results obtained for 3-MCA and DMN were fairly similar for both S-9 mixes, which suggested that the activation of both of these chemicals is not sensitive to the NADPH generating system. The DMN response increased with S-9 content, whereas the MCA mutagenic activity peaked near 60 ul/ml of S-9. The mutagenic activity of 2-AAF increased rapidly as the S-9 mix was increased and a maximum was reached at 80 ul/ml of S-9 content. Thus, all three chemicals could be effectively assayed with S-9 mix concentrations yielding approximately 60 - 80 ul/ml of S-9.

The biological equivalents of the spectrophotometric titrations performed earlier for establishing the isocitrate to NADP molar ratio were performed to see if NADPH utilization by the test chemicals would influence the chosen ratio In these experiments, the NADP and S-9 copncentrations were held constant at 1mM and 30 ul/ml respectively. The concentration of isocitrate was increased from 0 to 10 mM. AAF and MCA induced maximal mutant frequencies when the isocitrate concentration reached 2 mM; DMN appeared to require approximately 4 mM isocitrate. Higher concentrations of isocitrate were largely ineffective in increasing the mutant frequency and tended to cause greater toxicity. Therefore, a 5:1 molar ratio of isocitrate to NADP appeared to provide a saturating source of energy for the metabolic activation experiments.

Magnesium ions are required for the enzymatic conversion of NADP to NADPH and and many laboratories include Mg^{++} ions in the S-9 mix formulation. To explore the possible controlling effect of Mg^{++} on the activation process, 2 mM Mg^{++} was added to the medium in the isocitrate titrtation experiment for AAF and DMN. This was an increase of 5-fold over the content of Mg^{++} in Fischer's medium. For both chemicals, the mutant frequency was clearly decreased, and no change in the isocitrate requriement was noted. It appears that the addition of exogenous Mg^{++} may be detrimental to the activation of chemicals.

Using a different batch of S-9 and a cofactor solution composed of NADP, G-6-P, $MgCl_2$ and KCl, no serious toxicity was observed with water as a solvent control or with 3 ug/ml natulan up to an S-9 concentration of 15 to 20 ul/ml.

The results obtained suggest that an optimal S-9 mix can be designed by the approach described. Each batch of S-9 may require a somewhat different formulation. The optimal S-9 mix condition for the studies performed with NADP and isocitrate appears to be about 60 ul/ml S-9, 1mM NADP and 5 mM isocitrate. No significant differences were observed with a cofactor solution using NADP and G-6-P.

Studies on optimizing the S-9 formulation will continue. In addition, compounds are and will contiue to be tested for their mutagenic activity using the mouse lymphoma L5178Y assay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program (NTP) organizes and conducts a comprehensive interagency testing and research program focused on determining potential human health hazards due to environmental exposure to chemicals. This work supports these efforts by identifying the potential mutagenic activity of compounds which may be hazardous to man.

SRI INTERNATIONAL - Menlo Park, California
(NO1-ES-28038)

TITLE: Evaluation of Small Colony/Large Colony Phenomenon in Mouse Lymphoma Assay

CONTRACTOR'S PROJECT DIRECTORS: Ann D. Mitchell, Ph.D.
Colette J. Rudd, Ph.D.
William F. Blazak, Ph.D.

PROJECT OFFICER (NIEHS): William J. Caspary, Ph.D.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $283,391

PROJECT DESCRIPTION

OBJECTIVES: The L5178Y mouse lymphoma cell mutagenesis assay has been a useful in vitro tool for the assessment of the mutagenic potential of chemicals. One characteristic of this test is that the mutant colonies consist of large and small colonies. It has been reported that the small colonies have a high frequency of chromosomal aberrations. The objectives of this contract are to (1) investigate the causes of the bimodal size distribution of triflurothymidine resistant (TFT^r) colonies from control and mutagen treated cultures and (2) if chromosomal damage is confirmed to be closely correlated with small colony formation, develop a cost and time efficient screening system to measure both point mutations and chromosomal damage.

METHODS EMPLOYED: The major emphasis during this initial phase of the study consisted of isolating clones of chemically induced $TK^{-/-}$ cells to investigate the relationship of the bimodal distribution of colony sizes with cytogenetic damage, growth rates and thymidine kinase enzyme activity. Methods for preparing the cells for cytogenetic analysis were developed.

MAJOR FINDINGS AND PROPOSED COURSE: During the initial phase of this contract several cultures and subclones of the L5178Y mouse lymphoma $TK^{+/-}$ cell line 3.7.2C have been cytogenetically characterized. Both small and large TFT^r colonies have been isolated from cell cultures exposed either to the solvent (DMSO) or to methylmethanesulfonate (MMS); a total of 37 of these colonies are presently being evaluated for chromosome abnormalities. Techniques were developed for determining the number of viable cells in various sizes of colonies and characterizing their growth in agar and for obtaining sensitive measurements of the thymidine kinase enzyme activity in dispersed colonies or cell suspensions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recently, evidence has been accumulating on the association of cellular chromosomal abnormalities with an increased probability for tumor development. This project is designed to evaluate the possibility of obtaining information from the L5178Y mouse lymphoma cell mutagenesis assay which may reflect the relative ability of a chemical to cause chromosomal damage. If this additional knowledge can be gained with little or no additional cost, then the assay could prove to be a valuable prescreen for clastogenic chemcials.

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH, FDA
Jefferson, AR 72079
(YO1-ES-30104)

TITLE: CGT Rapid Test Response - Component 1: In Vitro Unscheduled DNA Synthesis (UDS) in Rat Hepatocyte Assay

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Daniel Casciano

PROJECT OFFICER (NIEHS): Judson W. Spalding, Ph.D.
Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: 1. December 31, 1981 (YO1-ES-20077)
2. December 31, 1982 (YO1-ES-30104)

CURRENT ANNUAL LEVEL: $138,210

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 40 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: Twenty-seven coded chemicals of priority interest to NTP were assigned and completed in the assay. These results are presently undergoing evaluation. This contract terminated November 30, 1982.

A new three-year contract was initiated on December 31, 1982. Forty coded chemicals will be assigned for test each year. These will include the bioassay-candidate chemicals for each current year as well as selected chemicals for retrospective evaluation of their genetic toxicity.

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.

1. MICROBIOLOGICAL ASSOCIATES - Rockville, Maryland
(NO1-ES-3-5021)

2. SRI INTERNATIONAL - Menlo Park, California
(NO1-ES-3-5022)

TITLE: Salmonella Mutagenesis Testing

CONTRACTOR'S PROJECT DIRECTORS: 1. Steve Haworth, Ph.D.
2. Kristien Mortelmans, Ph.D.

PROJECT OFFICER: Errol Zeiger, Ph.D., Supervisory Microbiologist
Cellular and Genetic Toxicology Branch

DATE CONTRACTS INITIATED: 1. January 15, 1983
2. January 15, 1983

CURRENT ANNUAL LEVEL: 1. $143,176
2. $166,123

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test environmental and commercial chemicals for mutagenicity using Salmonella typhimurium tester strains in 2 laboratories. Based on results in Salmonella chemicals will be 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-97, TA-98, TA-100, and TA-1535 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 in a modified protocol. Results are being entered directly into a minicomputer for transfer to the data-base system.

MAJOR FINDINGS AND PROPOSED COURSE: The laboratories have received their initial shipments of chemicals and have begun testing. It is anticipated that they will complete the testing of 150 chemicals this year. Information and data on specific chemicals will be provided to government personnel and the private sector on request.

Results of these tests will be routinely published in the NTP Bulletin and manuscripts will be written to present results of the chemicals in reviewed scientific journals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These contracts 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. CASE WESTERN RESERVE UNIVERSITY - Cleveland, Ohio
(NO1-ES-9-2136)

2. MICROBIOLOGICAL ASSOCIATES - Rockville, Maryland
(Formerly 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. Steve 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

CURRENT ANNUAL LEVEL: 1. $36,424 (Terminated)
2. $35,403 (Terminated)
3. $24,651 (Terminated)

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts was to test environmental and commercial chemicals for mutagenicity using Salmonella typhimurium tester strains in 3 laboratories. Based on results in Salmonella chemicals were 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 were used to test for mutagenicity using a modification of the Ames Salmonella microsome assay. All chemicals were incubated with tester strains in suspension prior to addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation was provided by liver S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats and Syrian Hamsters. All chemicals were tested blind at 5 doses, in triplicate, in each Salmonella strain. Also, all chemicals were retested at least one week following the first test. Results were entered directly into a minicomputer for transfer to the data-base system.

MAJOR FINDINGS AND PROPOSED COURSE: These contracts terminated in December, 1982 and January, 1983. The laboratories tested a total of 1003 test samples which encompassed 799 unique chemicals. New contracts have been awarded to SRI International and Microbiological Associates to continue microbial mutagenesis testing.

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 in reviewed scientific journals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These contracts allowed 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 are being used to assist in decisions regarding chemicals to be tested in subchronic and chronic toxicological tests.

PUBLICATIONS

Zeiger, E., Haworth, S., Speck, W., Mortelmans, K.: Phthalate ester testing in the National Toxicology Program's Environmental Mutagenesis Test Development Program. Environ. Health Perspect. 45: 99-101, 1982.

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

CURRENT ANNUAL LEVEL: 1. $188,593
2. $150,351
3. $178,485

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 60 environmental and commercial chemicals for mutagenicty 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 malanogaster 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 transloction 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 121 test samples to date. It is anticipated that an additional 50 samples will be tested this calendar year. Manuscripts are currently being written to present results of the initial chemicals in reviewed scientific journals. New contracts will be awarded at the expiration of the present contracts.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These contracts will allow the NIEHS to confirm Salmonella mutagenicity results in a whole animal system. Drosophila is capable of activating promutagens by means of an enzyme system similar to the mammalian activation system. Chemicals found to be mutagenic, therefore, will be given the highest priority for chronic toxicological and carcinogenesis testing by the National Toxicology Program.

1. COLUMBIA UNIVERSITY - New York, New York
(NO1-ES-90014)

2. LITTON BIONETICS, INC. - Kensington, Maryland
(NO1-ES-90013)

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

DATE CONTRACT INITIATED: 1. September 29, 1979
2. September 29, 1979

CURRENT ANNUAL LEVEL: 1. $338,321
2. $249,827

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 were required to standardize and validate a protocol before they were given chemicals for routine testing.

METHODS EMPLOYED: Chinese hamster ovary cells in culture are being used to test for the induction of chromosome aberrations and sister chromatid exchange, 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 are entered on standardized data forms and transferred to a computerized data base management system.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 135 samples (including 118 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 assay are positive in the cytogenetics assays. These contracts are in their final year (ending September 1983) and two new ones will be awarded to continue this effort at a testing rate of 40 chemicals/year per laboratory.

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.

PUBLICATIONS

Galloway, S.M., Bloom, A., Nakamura, F., Resnick, M.A., and Zeiger, E.: Development of a standard protocol for in vitro cytogenetic testing in CHO cells: Comparison of two laboratories and results for 22 compounds. (To be Submitted)

CHEMICAL PATHOLOGY BRANCH

CHEMICAL PATHOLOGY BRANCH

Summary Statement

Mission: During FY 83 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 10 comparative pathologists, 11 technicians, 2 secretaries and 1 stay-in-school student. Dr. Marilyn Wolfe, formerly of the New York State College of Veterinary Medicine at Cornell University, joined the Branch in January 1983. Dr. Morrow Thompson, formerly of the Chemical Industry Institute of Toxicology, joined Chemical Pathology in June 1983.

Accomplishments:

1. Management of Quality Assurance Program for the National Toxicology Program - During FY83 the Chemical Pathology Branch continued responsibility for evaluating the quality of pathology conducted in bioassays performed by the NTP. This included the review of 28 subchronic and 36 chronic bioassays during the first nine months of FY83 (Tables 1 and 2).

2. Implementation of TDMS - During FY83 the Toxicology Data Management System (TDMS) was implemented in three 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. incorporation of 15 interim evaluations in chronic studies for the purpose of defining chronic toxicity

    c. defining of criteria to be used for the diagnosis of proliferative lesions of the pituitary, thyroid, adrenal, pancreas and lung

    d. investigations on the relationship of specific tumor types to cause of death

    e. monitoring of the pathology aspects of the oral (ingestion) asbestos studies in rats being conducted at Hazleton Laboratories

f. evaluation of lesions produced by polybrominated biphenyls in rats and mice following a 6 month exposure with subsequent life time observation

g. evaluation of the comparative toxicity of C.I. direct blue 6 and benzidine in rats

h. evaluation of the pancreatic acinar cell lesions in over 2000 untreated control and vehicle male rats from 2 year studies

i. organized a scientific discussion with national and international scientists on classification and biological potential of pancreatic acinar cell lesions in rodents

j. 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. C. Wagner, Pneumoconiosis Research Unit, Medical Research Council, England

k. evaluation of the Strain A lung adenoma model to evaluate the carcinogenic potential of chemicals

l. studies on the pathogenesis of hepatotoxicity caused by furfural alcohol

m. clinical pathology assays for methyl bromide and hydrochlorothiazide bioassays

n. evaluation of immunological techniques to identify cell marker antigens in rodent tumors

o. investigations on the bioavailability of dioxin (TCDD) in soil

p. investigations on the toxicity of oil obtained from the Spanish Toxic Oil Syndrome.

4. Research Program - Independent studies and collaborative efforts with other laboratories in TRTP/NTP:

a. immunotoxic and myelotoxic effects of synthetic and natural estrogens, ochratoxin and other environmental chemicals

b. validate and develop clinical chemistry methods for use in pathology and toxicology methods

c. toxic effects of allyl isothiocyanate

d. toxic effects of 8-methoxypsoralen

e. evaluation of the effects of kepone on male reproductive capacity

f. investigation of the two-hit hypothesis of carcinogenesis

g. characterization of mononuclear cell leukemia of Fischer 344 rats.

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, 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. J. McLachlan - electron microscopy on CD-1 mouse fetal genital tract culture after prenatal treatment with DES

(3) Drs. Bachter, Weber, Dixon - effects of anti-cancer drugs on the immature testes - electron microscopy

(4) Dr. J. McLachlan - electron microscopy and special histologic techniques in the study of the effects of DES on the developing and adult reproductive tract

(5) Dr. I.P. Lee - electron microscopy support - animal testes

(6) Dr. Matsuda - electron microscopy support - tissue culture cells.

b. Laboratory of Pharmacology

(1) Drs. C. Schiller, Chapman, C. Shoaf - effects of TCDD on gut, liver, pancreas and lungs of rats, electron microscopy support

(2) Dr. B. Fowler - several projects involving EM support of studies on the ultrastructural effects of heavy metals on the kidney and liver

(3) Drs. J. Fouts, M. Coomes, Ms. R. Pohl - xenobiotic-metabolizing enzyme activity in skin and its response to environmental agents - electron microscopy support

(4) Drs. Philpot, Vanderslice - ultrastructural evaluation of microsomal pellets

(5) Dr. C. Shoaf - ultrastructural evaluation of lymph gland clots

(6) Dr. T. Devereaux - ultrastructural evaluation of rabbit lung cells.

c. Laboratory of Pulmonary Function and Toxicology

(1) Dr. R. DiAugustine - neuroendocrine epithelial cells of the guinea pig upper respiratory tract - electron microscopy support

(2) Dr. G. Hook - studies on the composition and ultrastructure of abnormal tubular myelin assembly in the lungs of patients with pulmonary alveolar proteinosis - electron microscopy support

(3) Dr. A. Brody - deposition and translocation of inhaled asbestos - electron microscopy support

(4) Dr. K. Sonstegard - neuroendocrine cells in rabbit fetal lung as a model for in-depth study - electron microscopy study

(5) Dr. A. Brody - deposition and translocation of tracheal crystalline silica - electron microscopy support.

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. (in press) (1983).

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b. Abstracts

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TABLE 1

SUBCHRONIC BIOASSAYS

CS-2
Tetranitromethane
AGE
Pentachloroanisole
Polysorbate 80
Ethylenediamine
Titanium Ferrocene
Toluene
Vinylcyclohexene Diepoxide (Gavage)
4,4'-Diamino-2,2'-stilbenedisulfonic Acid
C.I. Pigment Red 3
Mercuric Chloride
2,4-diaminophenol Dihydrochloride
HC Yellow 4
C.I. Direct Blue 15
3,3'-Dimethoxybenzidine
C.I. Pigment 23
Azodicarbonamide
NDEA
3,3'-Dimethylbenzidine
Tetrahydrofuran
Manganese Sulfate
Chloramphenicol
O-Nitroanisole
o-Benzyl-p-chlorophenol
C.I. Acid Red 114
DDEU
1-Amino-2,4-Dibromoanthroquinone

TABLE 2

CHRONIC BIOASSAYS

DGRE
HC Red 3
Tris(2-ethylhexyl) phosphate
Ethylene chlorohydrin
Pyridine (2)
Crocidolite
DMBA/TPA
Chlorodibromomethane
8-Hydroxyquinoline
DAPT
Chrysotile
Witch Hazel
Mirex (reread)
DMMPA (2)
Chrysotile
n-Butyl Chloride
Benzene (2)
p-Rosaniline (C.I. Basic Red 9)
Isopherone
Methyl Chloroform
Benzyl Chloride
Trichloroform
Dimethyl Hydrogenphosphite
1,3-Butadiene
Ethylene Oxide (NIOSH study)
Castor Oil
Trisodium Phosphate
H.C. Blue 2
Dimethylvinylchloride
THPS (tetrakis(hydroxymethyl)) phosphonium
t-Butyl Alcohol
Chloroform I (EPA study)
1,2-Epoxyhexadecane

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21006-03 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Chronic Exposure to Airborne Environmental Agents - Vinyl Chloride

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J.A. Moore Supervisory Veterinary Medical Officer TRTP 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

J.A. Moore	Supv. Veterinary Medical Officer	TRTP	NIEHS
E.E. McConnell	Veterinary Pathologist	CPB	NIEHS
G.A. Boorman	Veterinary Pathologist	CPB	NIEHS
J.K. Haseman	Statistician	BRAP	NIEHS

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 published. 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.

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.

PUBLICATIONS

Drew, R.T., Boorman, G.A., Haseman, J.K., McConnell, E.E., Busey, W.M., and Moore, J.A.: The effect of age and exposure duration on cancer induction by a known carcinogen. Toxicol. Appl. Pharmacol. 68:120-130, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21064-01 CPB

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioavailability of TCDD in Missouri Soil

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E.E. McConnell Veterinary Pathologist TRTP NIEHS

COOPERATING UNITS *(if any)*

Environmental Protection Agency
Laboratory of Molecular Biophysics, NIEHS

LAB/BRANCH

Chemical Pathology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TCDD (Dioxin) contaminated soil from two sites in Missouri is being investigated to determine the bioavailability in soil. Guinea pigs are being used in the investigation. Work is in progress.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigator and All Other Personnel Engaged on the Project:

E.E. McConnell	Veterinary Pathologist	CPB	NIEHS
J.A. Moore	Supv. Veterinary Medical Officer	TRTP	NIEHS
M. Harris	Biological Laboratory Technician	TRTP	NIEHS
J.D. Allen	Biological Laboratory Technician	TRTP	NIEHS
E. Haskins	Biological Laboratory Technician	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Hartley strain guinea pigs will be given various amounts of contaminated soil via gavage. TCDD spiked soil as well as TCDD in corn oil will be used as positive controls. Parameters being studied include clinical signs and histopathology.

MAJOR FINDINGS AND PROPOSED COURSE: Study will be started in June 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively large amounts (600-800 ppb) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been found in soil from two different locations in Missouri. It is extremely important to establish the bioavailability of TCDD in this soil to accurately determine the hazard to humans exposed to such an environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21065-01 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Chrysotile Exposure on Bone Marrow Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G.A. Boorman Veterinary Pathologist CPB NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Inc., Research Triangle Park, NC 27709

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Chrysotile asbestos fibers were administered via inhalation to mice for three days. Bone marrow cellularity, pleuripotent stem cells and macrophage granule progenitors were quantitated for twelve months following exposure. Bone marrow parameters were depressed at all time periods. Ultrastructural examination was used to confirm the deposition of the fibers in the centriacinar region of the lung.

Principal Investigator and All Other Personnel Engaged on the Project:

G.A. Boorman	Veterinary Pathologist	CPB	NIEHS
M.I. Luster	Immunologist	STB	NIEHS
M.P. Dieter	Physiologist	STP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The chrysotile asbestos exposure was used to determine if bone marrow and immune alterations(as reported in man with radiological evidence of asbestosis) could be produced in the mouse. The mice were six weeks old at the time of initial exposure and then held for 12 months with immune and bone marrow parameters measured at 0.5, 3, 6, 7, 9 and 12 months following exposure.

MAJOR FINDINGS AND PROPOSED COURSE: Depressed numbers of bone marrow pluripotent stem cells and macrophage granulocyte progenitors were found at all time periods. Immune alterations were not found. By 9 and 12 months the mice were showing mild leukopenia in addition to the bone marrow alterations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Canadian asbestos miners that appear clinically normal appear to have decreased circulating leukocytes. Similarly, Egyptian cement pipe cutters also show alterations in circulating leukocytes. These findings in mice suggest that inhalation of asbestos fibers can cause systemic bone marrow effects suggesting an additional toxic effect of asbestos fibers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21066-01 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Etiology of the Spanish Toxic Oil Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E.E. McConnell Veterinary Pathologist CPB NIEHS

COOPERATING UNITS *(if any)*

The Government of Spain
WHO Regional Office for Europe, Copenhagen, Denmark

LAB/BRANCH

Chemical Pathology Branch

SECTION

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Samples of suspect olive/rapeseed oil which were suspected of being part of the Spanish Toxic Oil Syndrome were given to guinea pigs and ducklings. The theory being tested was to rule out the presence of an obscure mycotoxin such as cytochalasin or trichothecene. The suspect oil samples did not produce disease in those laboratory species. Work is in progress.

Principal Investigator and All Other Personnel Engaged on the Project:

E.E. McConnell	Veterinary Pathologist	CPB	NIEHS
J.A. Moore	Supv. Veterinary Medical Officer	TRTP	NIEHS
M. Harris	Biological Laboratory Technician	TRTP	NIEHS
J.D. Allen	Biological Laboratory Technician	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Seven suspect oil samples were given by gavage to weanling guinea pigs and ducklings (1 week of age) by subcutaneous injection. Controls received pure olive oil or rapeseed oil. The animals were observed for 14 (ducks) or 30 (guinea pigs) days at which point they were killed and subjected to histopathologic examination. An additional study using mice will be conducted using a different suspect oil sample. This study is being coordinated by WHO and will be conducted using the same oil and protocol in Spain, the United Kingdom (MRC Laboratories) and the U.S. (NIEHS). Results from the three laboratories will be compared.

MAJOR FINDINGS AND PROPOSED COURSE: No adverse clinical signs or pathologic effects were observed with the first oil samples. It is suspected that these samples of oil were not part of the Spanish Toxic Oil Syndrome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: To date the etiologic factor responsible for sickness of >30,000 and death of >1000 Spaniards has not been determined. There is definite epidemiologic data which incriminates adulterated olive oil (with rapeseed oil, etc.) as the cause of the illness, but the specific etiologic factor in the oil has not been identified. The disease syndrome is felt to be related to endothelial damage. It would be extremely important to identify the etiologic factor so that such a disaster might be prevented in the future.

PUBLICATIONS

McConnell, E.E.: Investigations on the Etiologic Factor of the Spanish Toxic Oil Syndrome. International Working Group on Denatured Rape-Seed Oil Toxicology Syndrome, Madrid, March 21-25, 1983.

ARGONNE NATIONAL LABORATORY, ARGONNE, IL 60439
(222Y01-ES-20091)

TITLE: Refinement and Use of Peraino Rat Liver Tumor Model in Investigation of Hepatocarcinogenesis

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Carl Peraino, Ph.D.

PROJECT OFFICER (NIEHS): R. R. Maronpot, D.V.M., Head, Experimental Pathology, Chemical Pathology Branch, TRTP, NTP

DATE CONTRACT INITIATED: September 29, 1982

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: Refine the neonatal rat short-term in vivo liver tumor model described by Peraino (Carcinogenesis 1981:2, 463) to investigate mechanism of carcinogenesis. Utilize the refined model to test selected chemicals as initiators and promotors of hepatocarcinogenesis.

METHODS EMPLOYED: Neonatal rats will be dosed with known initiators and promotors to determine optimal dose response and timing of chemical administration. Effects of standard versus purified rodent diet on the liver tumor response will be determined. Multiple interim sacrifices will permit examination of the development and progression of preneoplastic alterations in the liver.

MAJOR FINDINGS AND PROPOSED COURSE: In-life portions of several studies are underway. Results from some of these studies will be available in FY 1984.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information gained from this research contract will help in the interpretation of liver tumor responses observed in conventional two-year rodent carcinogenesis tests and should provide basic insight into mechanism of carcinogenesis. The most frequent carcinogenic effect observed in NCI/NTP two-year carcinogenesis studies is the liver tumor response.

LITTON BIONETICS, INC., Kensington, MD 20895
(NO1-ES-3-5023)

TITLE: Refinement and Use of a Short-term In Vivo Rat Liver Tumor Model in Investigation of Mechanisms of Carcinogenesis

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Michael R. Moore, Ph.D.

PROJECT OFFICER (NIEHS): R. R. Maronpot, D.V.M., Head, Experimental Pathology, Chemical Pathology Branch, TRTP, NTP

DATE CONTRACT INITIATED: March 15, 1983

CURRENT ANNUAL LEVEL: $212,763.00

PROJECT DESCRIPTION

OBJECTIVES: Refine a short-term in vivo rat liver tumor model such as that described by Pitot (Nature 1978:27,456) and test selected chemicals using the refined model.

METHODS EMPLOYED: Rats will be administered known initiators and promotors in conjunction with partial hepectomy to determine optimal dose response and timing of chemical administration. Effects of standard rodent diet versus purified rodent diet, route of chemical administration, and sex of subsequent development of liver tumors will be determined. Early indicators of response such as histochemical foci of alteration will be assessed in addition to an actual tumor endpoint. Once the model has been refined, chemicals which have previously been tested in conventional two-year rodent carcinogenesis tests will be tested as initiators, promotors, and complete carcinogens in the short-term model.

MAJOR FINDINGS AND PROPOSED COURSE: In-life portions of several studies are underway. Results from some of these studies will be available in FY 1984.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information gained from this research contract will help in the interpretation of liver tumor responses observed in conventional two-year rodent carcinogenesis tests and should provide basic insight into mechanism of carcinogenesis. The most frequent carcinogenic effect observed in NCI/NTP two-year carcinogenesis studies is the liver tumor response.

BOARD OF REGENTS OF THE UNIVERSITY OF WISCONSIN SYSTEM, MADISON, WI 53706
(NO1-ES-3-5024)

TITLE: Refinement and Use of a Short-term in Vivo Rat Liver Tumor Model in Investigation of Mechanisms of Carcinogenesis

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Henry C. Pitot, M.D., Ph.D

PROJECT OFFICER (NIEHS): R. R. Maronpot, D.V.M., Head, Experimental Pathology, Chemical Pathology Branch, TRTP, NTP

DATE CONTRACT INITIATED: March 15, 1983

*CURRENT ANNUAL LEVEL: $169,853.00

PROJECT DESCRIPTION

OBJECTIVES: Refine a short-term in vivo rat liver tumor model such as that described by Pitot (Nature 1978:27,456) and test selected chemicals using the refined model.

METHODS EMPLOYED: Rats will be administered known initiators and promotors in conjunction with partial hepectomy to determine optimal dose response and timing of chemical administration. Effects of standard rodent diet versus purified rodent diet, route of chemical administration, and sex on subsequent development of liver tumors will be determined. Early indicators of response such as histochemical foci of alteration will be assessed in addition to an actual tumor endpoint. Once the model has been refined, chemicals which have previously been tested in the conventional two-year rodent carcinogenesis tests will be tested as initiators, promotors, and complete carcinogens in the short-term model.

MAJOR FINDINGS AND PROPOSED COURSE: In-life portions of several studies are underway. Results from some of these studies will be available in FY 1984.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information gained from this research contract will help in the interpretation of liver tumor responses observed in conventional two-year rodent carcinogenesis tests and should provide basic insight into mechanism of carcinogenesis. The most frequent carcinogenic effect observed in NCI/NTP two-year carcinogenesis studies is the liver tumor response.

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. - Herndon, Virginia 22070

NO1-ES-95618

TITLE: Pathology Support for the Carcinogenesis Testing Program (Task I)

CONTRACTOR'S PROJECT DIRECTOR: Dr. John F. Ferrell

PROJECT OFFICER (NIEHS): Dr. E.E. McConnell, Chief, Chemical Pathology Branch

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $379,671

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. Processed kidney and uterus from 65 mice some of which had adrenalectomies and received Estradiol via subcutaneous injection. The slides were delivered on October 2, 1982.

B. Twenty-three tissues from each of 28 mice which had received Allyl Isovalerate or corn oil via oral gavage were processed and the slides delivered on November 8, 1982.

C. Fifteen tissues from each of 35 mice which had received Didemethoyl Methoxychlor or Methoxychlor via subcutaneous injection were processed to H&E slides and delivered on December 20, 1982.

D. The contractor was requested to characterize a suspected "ITO cell" tumor from a control mouse from a chronic bioassay. Frozen fat stains, reticular stains and additional H&E stains have been performed. Ultrastructural evaluation of the tumor is in progress.

E. The contractor evaluated tissue sections from 100 Swiss mice administered Piperine for twenty-four months. His results were reported in a detailed histopathology report.

END

F. The contractor was requested to evaluate microscopically hematoxylin and eosin stained tissue sections from rats and hamsters which had received morpholine in their drinking water and nitrogen dioxide via inhalation. This work has been completed and a detailed report was submitted on November 29, 1982.

G. Dr. Larry Ackerman and Ms. Helen Cooke visited the Raffington Laboratory, Papanicolaou Cancer Research Center, Miami, Florida for the purpose of evaluating the histology capability of the laboratory on October 20, 1982. A report of their findings was submitted to Dr. Dexter Goldman on November 1, 1982. Dr. Proctor monitored necropsies on two occasions at Gulf South Research Institute.

H. The contractor was requested to process rodent tissues from a number of studies conducted at the National Institute of Environmental Health Sciences to hematoxylin and eosin slides. These studies were as follows:

1) Asbestos Study IV - The contractor prepared H&E stained slides from 14 tissues from each of the 14 mice exposed to asbestos in an inhalation study and the slides were submitted January 14, 1983. A second group was submitted on March 31, 1983.

2) Ochratoxin A (by gavage) - The contractor prepared hematoxylin and eosin stained slides of 23 tissues from each of 20 mice exposed to Ochratoxin A by gavage and submitted slides on March 23, 1983.

3) Organ Toxicity and Glutathione Dynamics Upon Acute and Prolonged Inhalation Exposure to Ethylene Oxide and Ethylene Dibrombide - The contractor prepared and examined tissues from 24 rats which had been exposed to Ethylene Oxide via inhalation for 48 six-hour exposures. A report was submitted to the Chemical Manager February 28, 1983. The contractor is currently preparing tissue sections for microscopic evaluation from 24 rats exposed to Ethylene Dibrombide for 1 six-hour exposures.

4) Preparation of Oil Red O Sections on Propyl Gallate and Geranyl Acetate - Selected tissues were prepared from animals from the chronic bioassay studies of Propyl Gallate and Geranyl Acetate and stained with an Oil Red O stain. These slides were submitted to NTP on January 28, 1983.

5) Phenytoin Study (1) - Hematoxylin and eosin stained slides were prepared of 14 tissues from each of 30 mice exposed to Phenytoin by gavage. Slides were submitted on March 31, 1983.

6) Potential <u>In Vivo</u> Interaction of Nitrogen Oxides with the Hetercyclic Amine 2,6-Dimethylmorpholine - The contractor was requested to prepare and examine hematoxylin and eosin stained tissue sections from rats used in a life time study of the carcinogenic potential of inhaled oxide of nitrogen in animals exposed to the heterocyclic amine 2,6-dimethylmorpholine in their drinking water. These evaluations are being performed by an EPL pathologist who will submit a detailed report upon completion of the study.

END

I. The contractor was requested to characterize a suspected "ITO cell" tumor from a control mouse from a chronic bioassay. Frozen fat stains, reticular stains and additional H&E stains have been performed. Ultrastructural evaluation of the tumor was completed and a report submitted to the NTP on March 23, 1983.

J. Carcinogenesis Bioassay of Coconut Oil - The contractor will be responsible for evaluating the pathology of the subject bioassay. The results of these evaluations will be completed in late FY 1983 and FY 1984.

K. Psoralen Bioassay Project - Histopathologic evaluation of all slides was completed on March 31, 1983. The results were entered onto the Toxicology Data Management System and forwarded to NCTR for input into the main frame computers and table generation.

L. Inventory of Liver Sections on Chronic Bioassay of Mirex - The contractor prepared a tissue inventory of the number of liver sections per rat in a chronic bioassay study of Mirex. A report of these findings was submitted to the NTP on January 31, 1983.

M. Evaluation of Pancreatic Lesions in Corn Oil Vehicle Control Rats - The contractor initiated a study to evaluate the relative amount of pancreatic tissue from vehicle control male rats from four chronic bioassay studies to determine the possible relationship to the number of proliferative acinar lesions present. A report of the findings will be submitted upon completion of the study. The four studies are Benzyl Acetate, Geranyl Acetate, Tetrachloroethylene and Methylene Chloride.

N. Review of Spleen and Liver from Sodium Dodecyl Sulfate - A pathologist reviewed the spleen and liver from female rats in a chronic bioassay study with Sodium Dodecyl Sulfate for the presence of mononuclear cell leukemia. A report of the findings was submitted to the NTP on February 16, 1983.

O. Pathology Review of Chloroform in Rats - Slides were received from the Stanford Research Institute from a chronic toxicity of Chloroform in drinking water. The project is currently being organized for review of the pathology with completion of the review currently scheduled for May 1983.

P. Pathology Data Coordinator - Ms. Janice Greenberg, EPL, was assigned the duties of NTP Pathology Data Coordinator on October 18, 1982, and has performed the following major tasks during FY 1983:

1) Update and maintain PWG schedule.

2) Distribution of materials and slides following PWG review.

3) Update and maintain the Tumor Pathology Section chronic bioassay schedule.

4) Maintain a centralized listing of NTP/BOA chemicals on chronic and subchronic tests at subcontractor laboratories.

5) Maintained a master schedule for monitoring the progress of chemicals on chronic bioassay tests.

6) Maintained Compound Information Summary Forms for subchronic toxicity test submitted to the Toxicologic Pathology Section.

7) Maintain schedule of interim kill chemicals.

8) Prepare compound status summary report for all chemicals to be terminated in FY 1983 for Deputy Director, NTP.

9) Prepare preminutes for Testing Status meetings.

10) Receive and distribute Early Death Individual Animal Data Records.

11) Prepare special report summarizing chronic Pathology Working Groups held during 1980, 1981 and 1982.

12) Prepare a subchronic Chemical Summary Report for the Chief, Chemical Pathology Branch.

The Pathology Data Coordinator will pick-up these same tasks as of June 1, 1983 for studies conducted under the Tracor Jitco, Inc. prime contract.

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.

CLEMENT ASSOCIATES, INC. - Arlington, Virginia 22209

NO1-ES-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: $442,221

PROJECT DESCRIPTION

OBJECTIVES: To provide the necessary professional support 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) participate in advisory groups, workshops, seminars and site visits; and (4) conduct specific pathology support projects as directed by the NTP such as preparation of study sets, investigation of problems related to NTP pathology activities and quality assurance of occasional chemicals.

METHODS EMPLOYED: The above methods 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: During the period October 1, 1982 to September 30, 1983 the contractor has been working on the following tasks:

A. Review of pathology and toxicology of the subchronic and interim sacrifice studies of t-butanol.

B. Ethylene oxide pathology quality assessment.

C. Special Pathology Working Group to review selected rat hepatoproliferative lesions.

D. Literature search of secondary sources in preparation of study set of nonneoplastic toxic lesions.

E. Chairing of Pathology Working Group meetings.

F. Participation in Pathology Working Group meetings.

G. Preparation of microslide study sets.

H. Preparation of journal articles.

I. Site visit to International Research and Development Corporation to review histology laboratory.

J. Literature search and review to investigate the usefulness of clinical pathology in toxicology studies.

K. Histopathological review of rats from the Malathion I and II and Malaoxon bioassays.

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.

END

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. - Herndon, Virginia 22070

NO1-ES-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: $408,229

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 chronic studies conducted under the prime contract (Tracor Jitco) and those conducted under the NTP Basic Ordering Agreement (BOA) and subchronic studies conducted under the NTP/BOA.

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, 1982 to September 30, 1983 the chemicals subjected to QA were :

Chronic Studies

Chemical Name	Chemical Number
n-Butyl Chloride	C06155
DMMPA (Mice)	C54740
Benzene (Rats)	C55276
Isophorone	C55168
Benzene (Mice)	C55276
NDEA	C55583
Benzyl Chloride	C06360
Trichlorfon	C54831
HC Blue 2	C54897
1,3-Butadiene	C50602
Castor Oil (Mice)	C55163
Chlorinated Trisodium Phosphate	C55754
Pyridine	C55301
Diallyphthalate	C50657
Chlorodibromomethane	C55254
Witch Hazel	C50544
Pyridine (Mice)	C55301
DGRE	C54966
Chrysotile & DMH (Males)	C05743/ C61234

Chemical Name	Chemical Number
DMBA/TPA (Mice)	C03918
Furan	C56202
Mirex	C06428
DMMPA (Rats)	C54740
Dimethyl Hydrogenphosphite	C54773
p-Rosaniline	C54739

Subchronic Studies

Chemical Name	Chemical Number
Azodicarbonamide (Mice)	C55981
Pentachloranisole	C56520
C.I. Direct Blue 15	C61290
3,3'-Dimethoxybenzidine	C99989
o-Nitroanisole	C60388
C.I. Pigment Red 23	C60377
Vinylcyclohexene Diepoxide (Gavage)	C60139
Azodicarbonamide	C55981
Mercuric Chloride	C60173
H.C. Yellow No. 4	C56019

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.

PROGRAM 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

The "National Toxicology Program (NTP) General Statement of Work for the Conduct of Acute, Fourteen Day Repeated Dose, Ninety Day Subchronic, and Two Year Chronic Studies in Rodents" was completely revised and issued as the Statement of Work for an RFP for potential Master Agreement holders qualified to conduct such studies. There were twenty-three responders including seventeen of the current nineteen Master Agreement awardees. It is anticipated that all reviews will be completed and Master Agreement awards will be made early in FY 84 for a period of five years subject to annual review. The Master Agreement will be readvertised again during Fiscal Year 84.

Nine chemicals arranged in seven bid packages were competed among the laboratories judged to be qualified under the Master Agreement during FY 82 with seven Task Order awards made to the four laboratories indicated in Table 1. Twelve chemicals, one per bid package, are being competed among the Master Agreement holders during FY 83.

Two interagency agreements were made in FY 82 as indicated in Table 2. One interagency agreement is in process for FY 83 for the study of three nickel compounds.

One support service contract award has been made in FY 83 for "Biochemical and Genetic Monitoring of Inbred Rodents." The award was for a three year effort to Research Triangle Institute (NO1-ES-3-8044) in the amount of $195,293. There were four respondees to this RFP announcement.

Two other support service RFP's have been issued during FY 83 as indicated below:

RFP NIH-ES-83-50016	Toxicology Research and Testing Program Health and Safety Support
	- 7 Respondees

RFP NIH-ES-83-50017 Good Laboratory Practices (GLP) Compliance Monitoring Support Resource

- 13 Respondees

It is anticipated that all reviews will be completed and awards made during FY 83.

The following support service RFP is in process with awards anticipated during the first quarter of FY 84:

RFP NIH-ES-83-50021 Rodent Foundation Colonies

Collaborative Services has also participated in: (1) The phase-in of Tracor Jitco subcontracts (see Table 3); (2) implementing, tracking, and monitoring of modifications to the Master Agreement, Task Order awards, and Interagency Agreements; and (3) debriefing of unsuccessful offerors.

Table 1. Chemicals Placed On Test During FY 82 Through Task Order Awards Under The Master Agreement

Bioassay Systems

1. Carisoprodol

Hazleton Laboratories America

1. Molybdenum trioxide

Litton Bionetics

1. p-nitrotoluene
2. 4-chloro-2-nitroaniline
3. Vinylidene fluoride
4. 2-mercaptobenzimidazole
5. Isobutyraldehyde

SRI International

1. Barium Chloride
2. Tetrahydrocannabinol

Table 2. Chemicals Placed On Test During FY 82 Through Interagency Agreements

Department of Energy/
Lovelace Inhalation Toxicology Research Institute

1. Talc

Department of Energy/
Brookhaven National Laboratory

1. Methylbromide

Table 3. Chemicals On Test That Were Phased Over To NTP Contracts From The Prime Contractor Tracor-Jitco

FY 82

Bioassay Systems

1. Hydroquinone

Southern Research Institute

1. CI Disperse Blue 1
2. H.C. Blue 2
3. H.C. Red 3
4. Nitrofurantoin
5. Pentachloroanisole
6. CI Acid Orange 3
7. Chlorowax 40
8. Chlorowax 500C
9. Dichlorvos

Papanicolaou Cancer Institute

1. 2,2-bis (bromomethyl) 1,3-propanediol
2. 2,3-dibromo-1-propanol
3. Isophorone
4. Glycidol

Microbiological Associates

1. Benzylalcohol
2. Methyl carbamate
3. d-limonene
4. alpha-methyl benzyl alcohol
5. succinic anbydide

Gulf South Research Institute

1. 2-butanone peroxide
2. Choramine
3. Coconut oil/diethanolamine
4. Diethanolamine
5. Glularaldehyde
6. Lauric acid/diethanolamine
7. p-nitrophenol
8. Aleic acid/diethanolamine

EGG Mason Research Institute

1. Boric acid
2. Bromoform
3. Bromodichoromethane
4. Butyl benzye phthalate
5. n-butyl chloride
6. chlorinated trisodium phosphate
7. Chlorodibromomethane
8. 1-2-dichloropropane
9. Diglycidylresorcinol ether
10. 8-hydroxyquinoline
11. Iodinated glyceral
12. 4,4'-methylenedianiline 2HCl
13. Numron
14. Pentachloronitrobenzene
15. Phenylbutazone
16. 2-biphenylamine HCl
17. CI Basic Red 9
18. bis (2-chloro-1-methylethyl) ether

FY 83

Battelle Columbus

1. p-dichlorobenzene
2. Malonaldehyde
3. Chlorpheniramine maleate
4. Pentachlorophenol Dowicide EC7
5. Pentachlorophenol Technical
6. N-phenyl-2-naphthylamine
7. o-phenylphenol
8. THPS (Tetrasis (hydroxymethyl) Phosphonium)
9. rotenone
10. Sodium fluoride
11. Xylenes
12. Benzene
13. 2,4,didlorophenol

Battelle Pacific Northwest

1. 1,3 butadiene
2. Propylene
3. Ethyl chloride
4. Allyl glycidyl ether
5. Epinephrine HCl
6. Methylene Chloride
7. Ethylbromide
8. alpha-chloroacetophenone
9. o-chlorobenzalmalonitrile
10. Propylene oxide
11. Methyl methacrylate
12. 1,2-epoxybutane
13. ethylene oxide
14. Tetrachloroethylene

SRI International

1. 8-methoxypsoralen
2. Hydrochlorothiazide
3. Furosemide
4. Diphenhydramine HCl

Litton Bionetics

1. Dimethyl methyl phosphonate

Physiological Research Laboratories

1. 2-amino-5-nitrophenol
2. Oxytetracycline hydrochloride
3. Nitrofurazone
4. Mercaptobenzothiazole
5. Nadidixic acid
6. Ephedrine sulfate
7. Tetracycline hydrochloride
8. Erythomycin stearate
9. Hexylresocinol
10. Phenylephrine hydroochloride
11. Methyldopa
12. 2-amino-4-nitrophenol

Midwest Research Institute

1. Tetranitromethane
2. vinyl toluene

Springborn Institute

1. Ampicillin trihydrate
2. Penicillin VKt
3. N,N-dimethylaniline
4. Benzofuran

Good Laboratory Practices Compliance

The NTP contractual studies are in compliance with the Food and Drug Administration (FDA) Good Laboratory Practices (GLP) Regulations (Federal Register, Dec. 22, 1978, Part II). This is assured through contractual arrangements and GLP monitoring of each laboratory's activities. The primary emphasis has been on animal studies for toxicology, carcinogenicity, reproductive and development toxicology. After procedures for cellular and genetic toxicology have been validated and a testing program established, these studies will also be carried out in compliance with GLP.

This year has seen the expansion of the Toxicology Data Management System (TDMS) to all new chronic toxicology and carcinogenicity studies. In collaboration with FDA, various aspects of this automated data collection system have been reviewed for GLP compliance, and appropriate suggestions are being addressed at this time.

Planning and Coordination

During FY 1983, the Planning and Coordination Group (PCG) has developed and implemented 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 been analyzed for special problem areas and updated for accuracy, consistency, and completeness.

PCG has developed a variety of programs during FY 1983 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 been developed for 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 to 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 Project Officers) also helped, during FY 1983, in the monitoring of the subcontract laboratories that had 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 Project Officers are responsible for these activities through supervision of the project officers and organizational planning and oversight of the monitoring function.

During 1982 and 1983, NTP phased out the prime contract with Tracor Jitco, Inc., and assumed full scientific and administrative management of all testing operations. The phase-over schedule was as follows:

Date of Phase-over	Laboratory	NIEHS Contract No.
5/1/82	Bioassay Systems Corporation	NO1-ES-28022
6/1/82	Southern Research Institute	NO1-ES-28024
7/1/82	Papanicolaou Cancer Res. Inst.	NO1-ES-28025
8/1/82	Microbiological Associates	NO1-ES-28026
9/1/82	Gulf South Research Institute	NO1-ES-28027
10/1/82	EG&G Mason Research Institute	NO1-ES-28033
11/1/82	Battelle-Columbus Laboratories	NO1-ES-38068
12/1/82	Battelle-Northwest Laboratories	NO1-ES-38061
1/1/83	SRI International	NO1-ES-38069
2/1/83	Litton Bionetics, Inc.	NO1-ES-38040
3/1/83	Physiological Research Labs.	NO1-ES-38041
4/1/83	Midwest Research Institute	NO1-ES-38042
5/1/83	Springborn Institute for Bio-Research, Inc.	NO1-ES-28014

HAZLETON RALTECH
Madison, WI
NCPO-5696-01

TITLE: Bioassay of Methylphenidate and Riddelliine

CONTRACTOR'S PROJECT DIRECTOR: Karen MacKenzie, Ph.D.

PROJECT OFFICER (NIEHS): Carrie E. Whitmire, Ph.D. (10/1/81-3/30/83)
Douglas Bristol, Ph.D. (4/1/83)

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $55,278

PROJECT DESCRIPTION

OBJECTIVES: Prechronic animal studies to determine specifications for chronic study in rodents.

METHODS EMPLOYED: Methylphenidate: dosed feed, rats and mice, special studies; Riddelliine: gavage, rats and mice, special studies and electron microscopic evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: To be determined by chemical manager.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: To be determined by chemical manager.

HAZLETON RALTECH
Madison, WI
NCPO-5696-02

TITLE: Bioassay of p-Nitroaniline and o-Nitroanisole

CONTRACTOR'S PROJECT DIRECTOR: Karen MacKenzie, Ph.D.

PROJECT OFFICER (NIEHS): Carrie E. Whitmire (10/1/81-3/30/83)
Douglas Bristol, Ph.D. (4/1/83)

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $69,428 Completed 4/30/83

PROJECT DESCRIPTION

OBJECTIVES: Prechronic animal studies to determine specifications for chronic study in rodents.

METHODS EMPLOYED: o-Nitroanisole: dose feed, rats and mice;
p-Nitroaniline: gavage, mice only.

MAJOR FINDINGS AND PROPOSED COURSE: To be evaluated by chemical manager.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: To be evaluated by chemical manager.

GULF SOUTH RESEARCH INSTITUTE - New Iberia, LA 70560
NO1-ES-05698-01

TITLE: Carcinogen Bioassay of Manganese Sulfate

CONTRACTOR'S PROJECT DIRECTOR: Mr. Ralph J. Wheeler

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $30,429

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the toxicity of manganese sulfate when administered in the diet and to establish data relevant to a subsequent chronic study.

METHODS EMPLOYED: Manganese sulfate was fed in the diet to both F344 rats and B6C3F1 mice of both sexes in 14-day repeated dose and 90-day subchronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: The 90-day study was completed and reported in FY83. Since this contract has been completed, the Chemical Manager now needs to evaluate the data and determine if a two-year bioassay is warranted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Manganese sulfate is used as fertilizer for vines and tobacco. The principal human exposure is from food. The daily estimated intake is estimated at 5 mg/day.

GULF SOUTH RESEARCH INSTITUTE - New Iberia, LA 70560
NO1-ES-05698-02

TITLE: Prechronic Studies of Tetrahydrofuran

CONTRACTOR'S PROJECT DIRECTOR: Mr. Ralph J. Wheeler

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $40,140

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the prechronic toxicity of tetrahydrofuran when administered by oral gavage and to establish data relevant for a subsequent chronic study.

METHODS EMPLOYED: The chemical was administered orally to both sexes of F344 rats and B6C3F1 mice by gavage in deionized water in 14-day repeated dose and 90-day subchronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: The 90-day study was completed and reported in FY83. Since this contract has been completed, the Chemical Manager needs to evaluate the data and determine if further testing is warranted.

Significance to Biomedical research and the Program of the Institute: Tetrahydrofuran has a very significant production record (4.5×10^6 kg/yr) with a potential for considerable human exposure. It is used in preparation of resinous and/or polymeric coatings for polyolefin films used to package foods. It appears as flavor components in coffee and irradiated apples and has been reported in raw and finished drinking waters.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
N01-ES-05700-01

TITLE: Carcinogenicity and Toxicity Studies on Toluene, Isoproterenol Hydrochloride and Dimetholdihydroxyethylene Urea (DDEU)

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: August 31, 1980

CURRENT ANNUAL LEVEL: $750,000

PROJECT DESCRIPTION

OBJECTIVES: To investigate the toxicity and carcinogenicity potential of selected chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute 14-day and/or 90-day toxicity tests were conducted via the inhalation and/or oral gavage routes. These prechronic tests were designed to characterize toxicity and permit the setting of doses for the 2-year chronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: Acute, 14-day and/or 90-day tests have been completed and reported. DDEU is currently in the 90-day phase and Toluene is in the chronic inhalation phase. Isoproterenol was cancelled for further tests following the acute inhalation phase for scientific reasons (improper route of exposure).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results of these studies will provide information about toxicity and carcinogenicity potential of these chemicals which may be useful in the assessment of their potential hazards to man.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
(N01-ES-05700-02)

TITLE: Toxicity Studies on Coumarin, 3,4-Dihydrocoumarin and 6-Methyl Coumarin

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: August 31, 1980

CURRENT ANNUAL LEVEL: $323,913

PROJECT DESCRIPTION

OBJECTIVES: To investigate the potential toxicity of three selected chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: All three chemicals were tested through acute, 14-day and 90-day toxicity tests (via gavage)

MAJOR FINDINGS AND PROPOSED COURSE: The prechronic tests have been completed and reported and the contract has been terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Results of these studies will provide further information about the toxicity of these chemicals and will permit selection of dose levels for any future chronic testing.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
N01-ES-05700-03

TITLE: Carcinogenicity and Toxicity Studies on Azodicarbonamide and Isobutyl nitrite

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: August 31, 1980

CURRENT ANNUAL LEVEL: $377,578

PROJECT DESCRIPTION

OBJECTIVES: To investigate the toxicity and carcinogenicity potential of these chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute and 14-day toxicity tests were performed via the inhalation route for Isobutyl nitrite and acute 14-day and 90-day tests via gavage and acute inhalation were performed for Azodicarbonamide. These tests were designed to permit establishment of dose levels for subsequent chronic tests.

MAJOR FINDINGS AND PROPOSED COURSE: Tests on Azodicarbonamide revealed a very low order of toxicity raising the possibility of poor absorption; further tests are being deferred pending planned inhalation pharmacokinetics. The acute and 14-day inhalation tests on Isobutyl nitrite have been completed and reported; chronic tests are scheduled.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results of these studies will provide information about the carcinogenic potential of each chemical in animals and may be helpful in the assessment of potential hazards to man.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
NO1-ES-05700-04

TITLE: Carcinogenicity and Toxicity Studies on Carvone, Resorcinol and Diethyl phthalate

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. C. Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: October 6, 1980

CURRENT ANNUAL LEVEL: $358,178

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the carcinogenicity and toxicity potential of three chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute, 14-day and 90-day (prechronic) studies were performed via oral gavage (Carvone and Resorcinol) and dosed-feed (Diethyl phthalate) to characterize toxicity and to set dose levels for subsequent chronic (2-year) tests.

MAJOR FINDINGS AND PROPOSED COURSE: Prechronic studies have been completed and reported and the chronic phase is in progress for Carvone and Resorcinol. No further testing was deemed necessary for Diethyl phthalate.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results from these studies will provide information on their carcinogenic potential in animals which may be useful in evaluating their potential hazards to man.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
NO1-ES-05700-05

TITLE: Carcinogenicity and Toxicity Studies on Mercuric chloride, Palladium chloride and Monochloroacetic acid (MCAA)

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: October 31, 1980

CURRENT ANNUAL LEVEL: $430,361

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the carcinogenicity and toxicity potential of there chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute, 14-day and /or 90-day prechronic tests via oral gavage were performed on these chemicals to characterize toxicity and permit the setting of doses for subsequent chronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: The prechronic tests have been completed and reported for these three chemicals. Chronic tests are in progress for Mercuric chloride and MCAA. Difficulties related to its physico-chemical properties were encountered in the acute and 14-day test phases; consequently further testing has been deferred for Palladium chloride.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Results from these studies will provide information regarding toxicity and carcinogenicity potential of these chemicals which may be useful in the assessment of their potential hazard to man.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
N01-ES-05700-06

TITLE: Carcinogenicity and Toxicity Studies on Chloramphenicol, 4,4-Diamino-2,2'-stilbenedisulfonic acid (DSDA) and Cadinene

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: October 31, 1980

CURRENT ANNUAL LEVEL: $402,000

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the carcinogenicity and toxicity potential of these chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute oral (via gavage) and 14-day and 90-day feeding studies were performed on these chemicals to characterize toxicity and permit the establishment of dose levels for the chronic tests.

MAJOR FINDINGS AND PROPOSED COURSE: The prechronic studies have been completed and reported. Chronic studies are currently in progress for DSDA, are scheduled for Chloramphenicol and were cancelled for Cadinene. Results from the 90-day tests for chloramphenicol suggest liver and thyroid toxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results from these studies will provide information on their carcinogenic potential in animals which may be useful in the assessment of their potential hazard to man.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
N01-ES-05700-07

TITLE: Carcinogen Bioassay of C.I. Direct Blue 218

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $707

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the toxicity of the chemical in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute (single dose via gavage), 14-day and 90-day subchronic feeding tests were performed on this chemical to characterize its short-term toxicity.

MAJOR FINDINGS AND PROPOSED COURSE: The acute and 14-day test phases have been completed and reported. The 90-day subchronic tests are in progress after which no further studies will be performed on this contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results from these studies can be used to evaluate the potential hazard of this chemical to man as well as permit the establishment of dose levels for future chronic tests.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION - Mattawan, Michigan 49071
N01-ES-05700-08

TITLE: Carcinogen Bioassay of Triamterene and Propantheline bromide

CONTRACTOR'S PROJECT DIRECTOR: Dr. D. Clifford Jessup

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $14,292

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the short-term toxicity of these chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute (single dose via gavage), 14-day and 90-day subchronic dietary studies were performed with Triamterene and 14-day and 90-day dietary studies on the second chemical.

MAJOR FINDINGS AND PROPOSED COURSE: The in-life phases have been completed and reports are in preparation for both chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Results from these studies will establish the short-term toxicity, if any, for these chemicals and provide information that may be useful in the assessment of their potential hazard to man. Data from these studies will also permit the establishment of dose levels for future chronic tests.

BROOKHAVEN NATIONAL LABORATORY, Upton, New York 11973
(Y01-ES-20087)

TITLE: Inhalation Study of Methyl Bromide

CONTRACTOR'S PROJECT DIRECTOR: Robert T. Drew, Ph.D.

PROJECT OFFICER (NIEHS): J. Fielding Douglas, Ph.D.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $245,270

PROJECT DESCRIPTION

OBJECTIVES: To investigate the toxicological and carcinogenic effect of Methyl Bromide in B6C3F1 mice.

METHODS EMPLOYED: Prechronic and chronic inhalation tests as described in the NTP general statement of work.

MAJOR FINDINGS AND PROPOSED COURSE: A repeated 2-week dose study will be completed in FY 1983, the 90-day prechronic study will be started in FY 1983, and the chronic study is scheduled to begin in early FY 1984.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Determination of the toxicity and potential carcinogenicity of this compound will be used in making regulatory decisions and promulgation of environmental standards.

LOVELACE INHALATION TOXICOLOGY RESEARCH INSTITUTE - Albuquerque, NM 87185
(1AG 222-Y01-ES-20088)

TITLE: Inhalation Study of Talc

CONTRACTOR'S PROJECT DIRECTOR: John A. Pickrell, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Sandra C. Brown, Ph.D., Chemist
Program Operations Branch, TRTP, NTP

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $285,098

PROJECT DESCRIPTION

OBJECTIVES: To investigate the toxicological effects of talc in rodents during chronic inhalation exposures.

METHODS EMPLOYED: A chronic inhalation test as described in the NTP general statement of work.

MAJOR FINDINGS AND PROPOSED COURSE: A preliminary four-week repeated dose study will be completed in FY 1983 which will be used to set dose levels for the chronic study to begin in FY 1984.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data will be used by regulatory agencies in determining policy regarding the test compound.

BIOASSAY SYSTEMS CORPORATION
Woburn, Massachusetts
(NO1-ES-28004-01)

TITLE: Toxicity Studies of Carisoprodol

CONTRACTOR'S PROJECT DIRECTOR: Indu Muni, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D, (From 4/1/83)

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $69,308

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for the chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of the chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE: Pharmacological (behavioral) range-finding tests were initiated in February, 1983. The subchronic toxicity test will start in June, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

LITTON BIONETICS, INC. - Rockville, Maryland 20850
N01-ES-28009-01

TITLE: Carcinogen Bioassay of Promethazine and Methdilazine

CONTRACTOR'S PROJECT DIRECTOR: Dr. Allan G. Manus

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $313,473

PROJECT DESCRIPTION

OBJECTIVES: To investigate the short-term toxicity of these chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Acute, 14-day repeated and 90-day subchronic studies were performed with Promethazine and 14-day and 90-day studies with Methdilazine. The chemicals were administered orally by gavage. Special studies consisting of hematology, sperm morphology, vaginal cytology and immunology were also performed at the termination of the 90-day subchronic phases for these chemcials.

MAJOR FINDINGS AND PROPOSED COURSE: All of the in-life phases for both chemicals have been completed and reports have been submitted with the exception of the immunology tests on Methdilazine. No further tests are scheduled under this contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data collected and submitted to the National Toxicology Program from these studies will enable that organization to assess the carcinogenic toxicologic potential of the chemical compounds under investigation. The data submitted are thus a part of the total input to the overall program of evaluation of suspect industrial carcinogens that the NTP has undertaken and may be useful in the assessment of their potential hazard to man.

LITTON BIONETICS, INC. - Rockville, Maryland 20850
N01-ES-28009-02

TITLE: Carcinogen Bioassay of 4-Chloro-2-nitroaniline and p-Nitrotoluene

CONTRACTOR'S PROJECT DIRECTOR: Dr. Allan G. Manus

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $220,504

PROJECT DESCRIPTION

OBJECTIVES: To investigate the short term toxicity of these chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Animals were exposed orally by gavage for 14-days to each of the two chemicals. Subsequently 90-day subchronic tests will be performed. Special studies consisting of hematology, methemoglobin and selected clinical chemistry determinations will be performed at termination.

MAJOR FINDINGS AND PROPOSED COURSE: The 14-day repeated dose tests for each chemical have been completed and reported and 90-day subchronic tests will follow. In addition to the above mentioned special studies, skin sensitization tests will be undertaken to investigate their potential for skin sensitization. No further studies beyond the 90-day subchronics are scheduled under this contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data collected and submitted to the National Toxicology Program from these studies will enable that organization to assess the carcinogenic and toxicologic potential of the chemical compounds under investigation. The data submitted are thus a part of the total input to the overall program of evaluation of suspect industrial carcinogens that the NTP has undertaken and may be useful in the assessment of their potential hazard to man.

LITTON BIONETICS, INC. - Rockville, Maryland 20850
N01-ES-28009-03

TITLE: Carcinogen Bioassay of Vinylidene Fluoride

CONTRACTOR'S PROJECT DIRECTOR: Dr. Allan G. Manus

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: August 30, 1982

CURRENT ANNUAL LEVEL: $101,913

PROJECT DESCRIPTION

OBJECTIVES: To investigate the short-term toxicity of the chemical in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Animals will be exposed by the inhalation route (whole-body exposures) in 14-day repeated dose and 90-day subchronic studies. Several special studies will be performed at the termination of the 90-day subchronic tests.

MAJOR FINDINGS AND PROPOSED COURSE: The 14-day repeated dose tests in both species have been completed and reported. The 90-day subchronic tests will be initiated as soon as doses are set by the program. No further tests beyond the subchronic phase are scheduled under this contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data collected and submitted to the National Toxicology Program from these studies will enable that organization to assess the carcinogenic and toxicologic potential of the chemical compounds under investigation. The data submitted are thus a part of the total input to the overall program of evaluation of suspect industrial carcinogens that the NTP has undertaken and may be useful in the assessment of their potential hazard to man.

LITTON BIONETICS, INC. - Rockville, Maryland 20850
N01-ES-28009-04

TITLE: Carcinogen Bioassay of 2-Mercaptobenzimidazole (2-MBI) and Isobutyraldehyde (IBA)

CONTRACTOR'S PROJECT DIRECTOR: Dr. Allan G. Manus

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $249,679

PROJECT DESCRIPTION

OBJECTIVES: To investigate the short-term toxicity of the two chemicals in F344 rats and B6C3F1 mice.

METHODS EMPLOYED: Animals will be exposed orally by gavage to 2-MBI and by inhalation to IBA in 14-day repeated dose and 90-day subchronic tests. Special studies of hematology, clinical chemistry, sperm morphology and vaginal cytology will be performed at the termination of each test.

MAJOR FINDINGS AND PROPOSED COURSE: The 14-day repeated dose tests for IBA have been completed and reported and under review by NTP for setting doses for the subsequent 90-day tests. The in-life 14-day phase on 2-MBI has been completed and the report is in preparation. No further tests beyond the 90-day phases are scheduled under this contract.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data collected and submitted to the National Toxicology Program from these studies will enable that organization to assess the carcinogenic and toxicologic potential of the chemical compounds under investigation. The data submitted are thus a part of the total input to the overall program of evaluation of suspect industrial carcinogens that the NTP has undertaken and may be useful in the assessment of their potential hazard to man.

SPRINGBORN INSTITUTE FOR BIORESEARCH
Spencerville, OH
(NO1-ES-28014)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals

CONTRACTOR'S PROJECT DIRECTOR: Richard A. Hiles, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D., Senior Project Officer (POB/TRTP)

DATE CONTRACT INITIATED: April 30, 1983

CURRENT ANNUAL LEVEL: $158,000 (Est.)

PROJECT DESCRIPTION

OBJECTIVES: To determine the chronic toxicity and carcinogenicity of 4 chemicals (N,N-Dimethylaniline, Benzofuron, Penicillin VK and Ampicillin Trihydrate).

METHODS EMPLOYED: Both sexes of F344 rats and B6C3F1 mice were dosed with chemical in a 104-week chronic study.

MAJOR FINDINGS AND PROPOSED COURSE: Chronic studies of each chemical are completed and final reports should be received early in FY'84. Results to be made public via standard NTP technical report.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Findings will be provided to regulatory agencies to assist them in making hazard and risk assessments.

SRI INTERNATIONAL - Menlo Park, CA 94025
(NO1-ES-28015)

TITLE: Bioassay Testing of Barium Chloride and Tetrahydrocannabinol

CONTRACTOR'S PROJECT DIRECTOR: William E. Davis, Jr.

PROJECT OFFICER (NIEHS): Sandra C. Brown, Ph.D., Chemist
Program Operations Branch, TRTP, NTP

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $405,458

PROJECT DESCRIPTION

OBJECTIVES: To investigate the toxicological effects of the test compounds in rodents and determine what, if any, further testing is required.

METHODS EMPLOYED: Prechronic testing of the two compounds, as described in the NTP general statement of work.

MAJOR FINDINGS AND PROPOSED COURSE: Subchronic testing of barium chloride will be completed in FY 1983 and that of tetrahydrocannabinol in FY 1984. Assessment of the results will be made to determine if chronic test should be conducted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data will be used by regulatory agencies in determining policy regarding the test compounds.

BIOASSAY SYSTEMS CORPORATION
Woburn, Massachusetts
(NO1-ES-28022)

TITLE: Bioassay Testing for Hydroquinone

CONTRACTOR'S PROJECT DIRECTOR: Indu Muni, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: April 30, 1982

CURRENT ANNUAL LEVEL: $196,029

PROJECT DESCRIPTION

OBJECTIVES: Determine from sub-chronic toxicity testing the toxicity, target organs and maximum tolerated doses for the chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of the chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE: In prechronic studies, hydroquinone was administered to rats and mice by gavage. A special study showing dermal absorption was also performed. The prechronic studies were finished in July, 1982. The chronic carcinogenicity (oral gavage) study was started in September, 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 22180
NO1-ES-28023-04

TITLE: Prechronic Studies of Molybdenum Trioxide

CONTRACTOR'S PROJECT DIRECTOR: William B. Coate, Ph.D.

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $180,046

PROJECT DESCRIPTION

OBJECTIVES: To determine the prechronic toxicity of molybdenum trioxide and to establish data relevant for a subsequent chronic study.

METHODS EMPLOYED: Both sexes of F344 rats and B6C3F1 mice were exposed to molybdenum trioxide via the inhalation route as an aerosol in 14-day repeated dose and 90-day subchronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: Both the 14-day and 90-day studies were completed and reported in FY83. The Chemical Manager needs to evaluate the data and determine if further testing is warranted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Molybdenum trioxide was chosen for study as a representative metal in a class study due to its high production, potential for significant human exposure and a suspicion of carcinogenicity.

SOUTHERN RESEARCH INSTITUTE
Birmingham, Alabama 35255
(NO1-ES-28024)

TITLE: Bioassay Testing for Disperse Blue 1, HC Blue 2, Nitrofurantoin, Rhodamine 6G, HC Red 3, Chlorowax 500 C, Acid Orange 3, Dichlorvos, Chlorowax 40, Roxarsone, and Pentachloroanisole

CONTRACTOR'S PROJECT DIRECTOR: Dr. J. D. Prejean

PROJECT OFFICER (NIEHS): William C. Eastin, Ph.D. (To 3/31/83)
Douglas W. Bristol, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: May 31, 1982

CURRENT ANNUAL LEVEL: $1,289,975

PROJECT DESCRIPTION

OBJECTIVES: The objective of this program is to investigate the chronic toxicity, including carcinogenic potential of 11 selected chemicals using Fischer 344 rats and B6C3F1 mice.

METHODS EMPLOYED: Two-year carcinogenesis bioassays of 11 chemicals in rats and mice are being performed by dosed feed or gavage administration. Chemical-induced lesions are evaluated by standard necropsy and histopathologic examinations.

MAJOR FINDINGS AND PROPOSED COURSE: The in-life phase of the chronic studies of Acid Orange 3, Chlorowax 40, Chlorowax 500 C, Disperse Blue 1, Dichlorvos, HC Blue 2, HC Red 3, Nitrofurantoin, and Rhodamine 6G have been completed. These studies have proceeded to histopathologic evaluation and report preparation. Roxarsone will undergo terminal sacrifice in June 1983. Pentachloroanisole will begin chronic study in FY83. The laboratory will continue histopathology and report preparation and initiate the chronic study of pentachloroanisole.

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.

PAPANICOLAOU CANCER RESEARCH INSTITUTE
Miami, Florida
(NO1-ES-28025)

TITLE: Bioassay Testing of Trichloroethylene, Isophorone, Glycidol, 2,2-Bis (Bromomethyl)-1,3-Propanediol, 2,3-Dibromo-1-Propanol

CONTRACTOR'S PROJECT DIRECTOR: Fred A. Bock, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D, (To 3/31/83)
Douglas Bristol, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: July 15, 1983

CURRENT ANNUAL LEVEL: $793,392

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Trichloroethylene: This chemical was tested in chronic carcinogenicity (gavage) studies in both mice and several strains of rats. Chronic testing was finished in 1981/1982. Report writing is in progress.

2. Isophorone: This chemical was administered by oral gavage to rats and mice in a chronic carcinogenicity test. Testing was completed in February, 1982. Histopathology is complete with report writing to follow.

3. Glycidol: This chemical is currently being tested by oral gavage in rats and mice in a chronic carcinogenicity test. Terminal sacrifice is scheduled for August, 1983.

4. 2,3-Dibromo-1-Propanol: This chemical was administered by skin paint to rats and mice in a chronic carcinogenicity test. Because of a viral disease problem, the test was terminated early (January, 1983). Histopathology is in progress.

5. 2,2-Bis (Bromomethyl)-1,3-Propanediol: The subchronic (skin paint) study of BMP was completed in March, 1983. Histopathology is in progress with a chronic carcinogenicity test tentatively scheduled for December, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

MICROBIOLOGICAL ASSOCIATES, INC.
Bethesda, Maryland
(NO1-ES-28026)

TITLE: Bioassay Testing of D-Limonene, Succinic Anhydride, Alpha-Benzyl Alcohol, Benzyl Alcohol, and Methyl Carbamate.

CONTRACTOR'S PROJECT DIRECTOR: Marshall Dinowitz, Ph.D.

PROJECT OFFICER (NIEHS): John A. Quest, Ph.D. (POB/TRTP)

DATE CONTRACT INITIATED: August 2, 1982

CURRENT ANNUAL LEVEL: $456,896

PROJECT DESCRIPTION

OBJECTIVES: To determine the chronic toxicity and carcinogenicity of each chemical.

METHODS EMPLOYED: Both sexes of F344 rats and B6C3F1 mice were dosed with chemical in a 104-week chronic study.

MAJOR FINDINGS AND PROPOSED COURSE: Chronic studies of each chemical are in progress. Results to be made public via standard NTP technical report.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Findings will be provided to regulatory agencies to assist them in making hazard and risk assessments.

GULF SOUTH RESEARCH INSTITUTE - New Iberia, LA 70560
NO1-ES-28027

TITLE: Bioassay Testing of 2-Butanone Peroxide, Chloramine, Coconut Oil Acid/DEA, Diethanolamine, Glutaraldehyde, Lauric Acid/DEA, p-Nitrophenol and Oceic Acid/DEA

CONTRACTOR'S PROJECT DIRECTOR: Mr. Ralph J. Wheeler and Milton R. Hejtmancik, Jr., Ph.D.

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: Phased-over from Tracor Jitco Subcontracts on September 1, 1982

CURRENT ANNUAL LEVEL: $1,638,599.

PROJECT DESCRIPTION

OBJECTIVES: To determine the chronic toxicity and/or carcinogenicity of these chemicals when administered to the F344 rat and B6C3F1 mouse of both sexes (the Swiss mouse is used for the p-nitrophenol study). In addition, to report the results from the two-year bioassay testing of t-butanol, Castor Oil, 1,2-epoxyhexadecane, ethylene, glycol monoethyl ether, gilsonite, trichlorfon, Witch Hazel and xylene sulfonic acid, the testing of which had already been completed.

METHODS EMPLOYED: A variety of routes was used to administer the test chemical and these were: Dosed feed - Castor Oil, gilsonite and xylene sulfonic acid; Drinking water - t-butanol and chloramine; Gavage - trichlorfon, diethanolamine and ethylene glycol monoethyl ether and; Skin paint - Witch Hazel, coconut oil/DEA, lauric acid/DEA, oleic acid/DEA, glutaraldehyde, 2-butanone peroxide, 1,2-epoxyhexadecane and p-nitrophenol. All chemicals were administered to both sexes of the F344 rat and the B6C3F1 mouse with the exception of p-nitrophenol, in which only the Swiss mouse was used.

MAJOR FINDINGS AND PROPOSED COURSE: None of these studies have been reported out to date. The following studies have been completed since the inception of this contract: diethanolamine, glutaraldehyde, coconut oil/DEA, oleic acid/DEA, lauric acid/DEA and 2-butanone peroxide. Complete the two studies in progress (chloramine and p-nitrophenol) and report out the results of the others for which testing is complete.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The chemicals listed have widespread usage as soaps, detergents, disinfectants, pesticides and fungicides. Thus, their potential for exposure is great.

EG&G MASON RESEARCH INSTITUTE
Worcester, Massachusetts
(NO1-ES-28033)

TITLE: Bioassay Testing of Bromodichloromethane, Bromoform, Iodinated Glycerol, n-Butyl Chloride, Phenylbutazone, Boric Acid and Chlorinated Trisodium Phosphate.

CONTRACTOR'S PROJECT DIRECTOR: Herman Lilja, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $136,877

PROJECT DESCRIPTION

OBJECTIVES: Determine from sub-chronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Boric Acid: Chronic carcinogenicity testing was completed in April, 1983. Histopathology currently in progress with report writing to follow. Mice received the test compound in the diet.

2. Bromodichloromethane: Chronic carcinogenicity test is in progress; terminal sacrifice is scheduled for June, 1983. Rats and mice received the test compound by gavage.

3. Bromoform: This compound was administered to rats and mice by gavage. The chronic carcinogenicity test was completed in March, 1983; histopathology is in progress with report writing to follow.

4. n-Butyl Chloride: This compound was tested in a chronic carcinogenicity test; rats and mice received the compound by oral gavage. The chronic test was completed in March, 1983; histopathology is in progress.

5. Chlorinated Trisodium Phosphate: This compound was administered to rats and mice by gavage. The chronic carcinogenicity test was completed in June, 1982; histopathology is in progress.

6. Iodinated Glycerol: This compound was administered to rats and mice by oral gavage. The chronic carcinogenicity test is due to be completed in May, 1983.

7. Phenylbutazone: This compound was administered to rats and mice by oral gavage. The chronic carcinogenicity study is in progress and is scheduled for terminal sacrifice in September, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

LITTON BIONETICS, INC. - Rockville, Maryland 20850
N01-ES-38040

TITLE: Carcinogenesis Bioassay

CONTRACTOR'S PROJECT DIRECTOR: Dr. Allan G. Manus

PROJECT OFFICER (NIEHS): Marcelina B. Powers, D.V.M., M.S.

DATE CONTRACT INITIATED: February 1, 1983 (Date of Phase-over from Tracor Jitco Subcontract)

CURRENT ANNUAL LEVEL: $591,355

PROJECT DESCRIPTION

OBJECTIVES: To investigate the carcinogenicity potential of 18 industrially important chemicals in rats and mice.

METHODS EMPLOYED: Prechronic tests utilizing single dose acute, 14-day repeated dose and/or 90-day subchronic studies were conducted to establish dose levels for subsequent 2-year chronic studies. Routes of administration included oral gavage, dietary, and dermal (skin paint).

MAJOR FINDINGS AND PROPOSED COURSE: Chronic studies for 12 chemicals (Caprolactam, Bisphenol A, Aminoundecanoic acid, 1,4-diamino-2,6-dichlorobenxene, Melamine, Diallyl phthalate, Toluene diisocyanate, Tris(2-ethyl hexyl)phosphate, Ethylene chlorohydrin, Dimethyl morpholino phosphoramidate, Dimethyl hydrogen phosphite and Dimethyl vinyl chloride) have been completed for one or both species and reported by NIEHS. Histopathology is in progress for four chemicals (4-Vinyl cyclohexane, 3-Chloro-2-methylpropene, Diesel fuel marine and JP-5 Navy fuel); the chronic phase is in progress for one chemical (Dimethyl methyl phosphonate) and cancelled for the 18th chemical (4,4-Diphenylmethane diisocyanate).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data collected from these studies will enable NTP to assess the carcinogenic potential of these chemicals in rodents which may be useful in the evaluation of their potential hazard to man.

PHYSIOLOGICAL RESEARCH LABORATORIES
Division of Medtronic, Inc.
Minneapolis, Minnesota 55433
(NIH-NO1-ES-38041)

TITLE: Bioassay Testing of Various Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Morris J. Cowan

PROJECT OFFICER (NIEHS): Ronald L. Melnick, Ph.D., Chemist, Carcinogenesis and Toxicological Evaluation Branch

DATE CONTRACT INITIATED: March 1, 1983

CURRENT ANNUAL LEVEL: $1,068,430.00

PROJECT DESCRIPTION

OBJECTIVES: To determine the chronic toxicity including carcinogenic potential, of 12 chemicals in male and female Fischer 344 rats and B6C3F1 mice. Separate studies are being conducted for five anti-bacterial agents (tetracycline, oxytetracycline, erythromycin, nitrofurazone, nalidixic acid); three cardiovascular agents (phenylephrine, ephedrine, methyldopa); two cosmetic dyes (2-amino-4-nitrophenol, 2-amino-5-nitrophenol); and two miscellaneous chemicals (hexylresorcinol; mercaptobenzothiazole).

METHODS EMPLOYED: Two-year carcinogenesis bioassays of 12 chemicals in rats and mice are being performed by dosed feed or gavage administration. Chemical induced lesions are evaluated by standard necropsy and histopathologic examinations.

MAJOR FINDINGS AND PROPOSED COURSE: The two-year exposures to ephedrine sulfate, phenylephrine HCL, oxytetracycline HCl, erythromycin stearate, tetracycline HCl, 2-amino-4-nitrophenol, hexylresorcinol, and nalidixic acid have been completed. These studies are in various stages of histopathologic evaluation. Special studies to examine ophthalmic toxicity in phenylephrine treated mice and rats were conducted with no chemical related effects observed. Determinations of blood prolactin levels in methyldopa treated female rats is ongoing. Special opthalmic examinations were conducted in rats and mice of the erythromycin stearate, hexylresorcinol, and 2-amino-5-nitrophenol studies to define the nature and extent of ocular opacities observed in treated and control animals. It is intended to continue performing the carcinogenesis bioassay exposure activities for the four remaining studies and to conduct each terminal sacrifice at its scheduled time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many of the chemicals being tested in the rodent carcinogenesis bioassay program are of vital importance to human welfare. Some of these compounds provide for the maintenance of man's health as pharmaceutical chemotherapeutics. It is the goal of the contractor to provide experimental data of high scientific credibility that can be used to relate tumor incidence resulting from chemical exposure in animals to carcinogenic hazard to man.

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64110
(NO1-ES-38042)

TITLE: Bioassay Testing of Vinyl Toluene and Tetranitromethane

CONTRACTOR'S PROJECT DIRECTOR: James Cholakis, Ph.D.

PROJECT OFFICER (NIEHS): Joseph H. Roycroft, Ph.D.

DATE CONTRACT INITIATED: April 1, 1983

CURRENT ANNUAL LEVEL: Estimated $700,000

PROJECT DESCRIPTION

OBJECTIVES: To perform two-year chronic bioassay testing of two compounds to determine potential toxicity and carcinogenicity.

METHODS EMPLOYED: Inhalation exposure 6 hrs/day, 5 days/week, for 103 weeks, followed by sacrifice and histopathological evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: No significant findings reported to date. Each chronic study will be completed, and resulting findings reported.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Determination of the toxicity and potential carcinogenicity of the two compounds will be used in making regulatory decisions and promulgation of environmental standards.

BATTELLE MEMORIAL INSTITUTE, PACIFIC NORTHWEST LABORATORIES
Richland, Washington 99352
(NO1-ES-38061)

TITLE: Chemical Testing of Various Chemicals: Methyl Methacrylate, 1,2-Epoxybutane, Ethylene Oxide, Tetrachloroethylene, Methylene Chloride, Ethyl Bromide, α-Chloroacetophenone, o-Chlorobenzalmalononitrile, Ethyl Chloride, Allyl Glycidyl Ether, and Epinephrine-HCl

CONTRACTOR'S PROJECT DIRECTOR: William J. Clarke, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Joseph H. Roycroft, Ph.D.

DATE CONTRACT INITIATED: November 29, 1982 (Phased over from Tracor Jitco Subcontract)

CURRENT ANNUAL LEVEL: $2,527,768

PROJECT DESCRIPTION

OBJECTIVES: To perform two-year chronic bioassay testing of 11 compounds (described above) to determine potential toxicity and carcinogenicity.

METHODS EMPLOYED: Inhalation exposure 6 hrs/day, 5 days/week for 103 weeks, followed by sacrifice and histopathological evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: Three chemicals--Propylene, Propylene Oxide, and 1,3 Butadiene--have been completed under Tracor Jitco Subcontract, and are now in the peer review process and will be reported under this contract. The other chemicals are in various stages of testing. Each chronic study will be completed, and resulting findings reported.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Determination of the toxicity and potential carcinogenicity of the compounds will be used in making regulatory decisions and promulgation of environmental standards.

SRI INTERNATIONAL - Menlo Park, CA 94025
(NO1-ES-38069)

TITLE: Bioassay Testing of Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Mr. William E. Davis, Jr.

PROJECT OFFICER (NIEHS): Sandra C. Brown, Ph.D., Chemist
Program Operations Branch, TRTP, NTP

DATE CONTRACT INITIATED: January 1, 1983

CURRENT ANNUAL LEVEL: $532,911

PROJECT DECRIPTION

OBJECTIVES: This contract is for the continuation of ongoing chronic toxicity studies of four chemicals--Furosemide, 8-Methoxypsoralen, Diphenhydramine HCl, and Hydrochlorothiazide--previously managed by Tracor Jitco under subcontract. The studies will be completed and provide information about the toxicological effects of the test compounds in rodents.

METHODS EMPLOYED: Chronic toxicity tests as described in the NTP general statement of work.

MAJOR FINDINGS AND PROPOSED COURSE: The testing phase will be completed for three of the compounds in FY 1983 and for the fourth in FY 1984. Results will be made public via the standard NTP procedures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Data will be used by regulatory agencies in determining policy regarding the test compounds.

TRACOR JITCO, INC. Rockville, Maryland 20852
(NO1-ES-43350)

TITLE: Bioassay Prime Contract

CONTRACTOR'S PROJECT DIRECTOR: Stephen A. Olin, Ph.D.

PROJECT OFFICER (NIEHS): J. Fielding Douglas, Ph.D.

DATE CONTRACT INITIATED: March 1, 1974

CURRENT ANNUAL LEVEL: Approximately $5,000,000

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, 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.

METHODS EMPLOYED: Tracor Jitco has supported bioassay and toxicological experiments on many chemicals in various phases of study.

MAJOR FINDINGS AND PROPOSED COURSE: All laboratories were phased over to NIEHS at a rate of one per month, so that Tracor Jitco had no subcontractors as of May 31, 1983. Tracor Jitco has been given a no-cost extension to December 31, 1983, to complete the close-out of all subcontracts and other miscellaneous functions. The findings from the carcinogenic and other toxicological studies are updated under individual laboratory headings. The proposed course will be to complete all financial aspects and reporting of the contract by December 31, 1983. Technical aspects for each laboratory have been phased over to NIEHS, being completed by May 31, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Significance can be found in the individual laboratory reports.

EG&G MASON RESEARCH INSTITUTE
Worcester, Massachusetts
(NO1-ES-95619-01)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for 2,6-Xylidine, Pentaerythritol Tetranitrate and HC Yellow 4

CONTRACTOR'S PROJECT DIRECTOR: Herman Lilja, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: June 30, 1980

CURRENT ANNUAL LEVEL: $418,570

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. 2,6-Xylidine: Prechronic phase finished in October, 1981. No further testing required. Final report in process.

2. Pentaerythritol Tetranitrate: Prechronic phase finished in September, 1981. Chronic test (dosed feed) started in January, 1982.

3. HC Yellow 4: Prechronic phase included comparative (feed vs. dermal) toxicity. Prechronic phase finished in October, 1982. Chronic test (dosed feed) started in March, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

EG&G MASON RESEARCH INSTITUTE
Worcester, Massachusetts
(NO1-ES-95619-02)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for Curcumin, Chlorpromazine Hydrochloride and 4-Hydroxyacetanilide

CONTRACTOR'S PROJECT DIRECTOR: Herman Lilja, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: August 31, 1980

CURRENT ANNUAL LEVEL: $279,194

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Chlorpromazine Hydrochloride: Prechronic studies were finished in February, 1982. NTP determined that there was no need for a chronic study.

2. 4-Hydroxyacetanilide: Prechronic studies were completed in January, 1982. A chronic carcinogenicity study (dosed feed) in rats and mice was started in July, 1982.

3. Turmeric, Oleoresin: (Replaced Curcumin): The subchronic (dosed feed) study was finished in October, 1982. This study is being evaluated; a chronic carcinogenicity study is scheduled for October, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

EG&G MASON RESEARCH INSTITUTE
Worcester, Massachusetts
(NO1-ES-95619-03)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for 2,4-Diaminophenol Hydrochloride, 1-Amino-2,4-Dibromoanthraquinone, Quercetin and Probenicid

CONTRACTOR'S PROJECT DIRECTOR: Herman Lilja, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D, (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: July 31, 1980

CURRENT ANNUAL LEVEL: $776,975

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. 1-Amino-2,4-Dibromoanthraquinone: Prechronic studies were finished in January, 1983. A chronic carcinogenicity (dosed feed) study in rats and mice is scheduled for June, 1983.

2. 2,4-Diaminophenol Dihydrochloride: Prechronic studies were completed in May, 1982. A chronic carcinogenicity (gavage) study in rats and mice was started in September, 1982.

3. Probenicid: Prechronic studies were finished in December, 1981. A chronic carcinogenicity (gavage) study in rats and mice was started in May, 1982.

4. Quercetin: No prechronic studies were carried out with quercetin. A chronic carcinogenicity (dosed feed) study was started in rats in June, 1982. Three dose levels were employed with two interim sacrifices.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

EG&G MASON RESEARCH INSTITUTE
Worcester, Massachusetts
(NO1-ES-95619-04)

TITLE: Carcinogenicity and Toxicity Studies in Rodents (Nitrobenzene, Titanium Ferrocene and Hexachloroethane)

CONTRACTOR'S PROJECT DIRECTOR: Herman Lilja, Ph.D.

PROJECT OFFICER (NIEHS): Dexter S. Goldman, Ph.D. (To 3/31/83)
John A. Quest, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: August 30, 1980

CURRENT ANNUAL LEVEL: $560,421

PROJECT DESCRIPTION

OBJECTIVES: Determine from subchronic toxicity testing the toxicity, target organs and maximum tolerated doses for each chemical in rats and mice. Determine from a chronic (2-year) study the carcinogenic potential of each chemical.

METHODS EMPLOYED: Gavage, skin paint, dosed-feed administration as required. Full or modified histopathology as required.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Hexachloroethane: The subchronic studies were finished in November, 1981; a chronic carcinogenicity (gavage) study in rats was started in April, 1982.

2. Nitrobenzene: Subchronic studies of the toxicity of nitrobenzene (gavage and dermal) were finished in March, 1983. The chronic carcinogenicity study is scheduled to begin in July, 1983.

3. Titanium Ferrocene: The prechronic studies were completed in July, 1982. A chronic carcinogenicity (gavage) study in rats and mice was started in February, 1983.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NTP bioassays are conducted by contractors to the NTP. Results of these tests are used by the referring regulatory agencies according to their mandates.

MICROBIOLOGICAL ASSOCIATES, INC.
Bethesda, Maryland
(NO1-ES-95650-01)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for Sodium Azide, Tris-2 (Chloroethyl) Phosphate and D,L-Amphetamine Sulfate

CONTRACTOR'S PROJECT DIRECTOR: Marshall Dinowitz, Ph.D.

PROJECT OFFICER (NIEHS): John A. Quest, Ph.D. (POB/TRTP)

DATE CONTRACT INITIATED: September 29, 1980

CURRENT ANNUAL LEVEL: $516,256

PROJECT DESCRIPTION

OBJECTIVES: To determine the chronic toxicity and carcinogenicity of each chemical.

METHODS EMPLOYED: Both sexes of F344 rats were dosed with Sodium Azide, and both sexes of F344 rats and B6C3F1 mice were dosed with Tris-2 (Chloroethyl) Phosphate and D,L-Amphetamine Sulfate in a 104-week chronic study.

MAJOR FINDINGS AND PROPOSED COURSE: Chronic studies of each chemical are in progress. Results to be made public via standard NTP technical report.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

Findings will be provided to regulatory agencies to assist them in making hazard and risk assessments.

SOUTHERN RESEARCH INSTITUTE
Birmingham, Alabama 35255
(NO1-95651-01)

TITLE: Carcinogenicity and Toxicity Studies in Laboratory Animals for Furan, Furfuryl Alcohol, Furfural, Gamma-Butyrolactone, Benzaldehyde, and Hexachlorocyclopentadiene

CONTRACTOR'S PROJECT DIRECTOR: Dr. J. D. Prejean

PROJECT OFFICER (NIEHS): William C. Eastin, Ph.D. (To 3/31/83)
Douglas W. Bristol, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: June 30, 1980

CURRENT ANNUAL LEVEL: $849,357

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. 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 standard chronic protocol on furan, and furfural was expanded to include a series of special studies.

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 and furfural were performed. The Toxicology Data Management System is being used to capture data in the chronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: Chronic studies on furan, furfural, gamma-butyrolactone, and benzaldehyde are in progress.

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
(NO1-ES-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 DIRECTOR: Dr. J. D. Prejean

PROJECT OFFICER (NIEHS): William C. Eastin, Ph.D. (To 3/31/83)
Douglas W. Bristol, Ph.D. (From 4/1/83)

DATE CONTRACT INITIATED: September 30, 1980

CURRENT ANNUAL LEVEL: $918,958

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 chronic 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 is being used to collect chronic data.

MAJOR FINDINGS AND PROPOSED COURSE: Chronic testing of polysorbate 80, ethylene glycol, CI Pigment Red 3, and CI Pigment Red 23 is in progress.

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.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 22180
NO1-ES-95655-01

TITLE: Carcinogen Bioassay of 1,2,3-Trichloropropane

CONTRACTOR'S PROJECT DIRECTOR: Borge M. Ulland, D.V.M.

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: August 31, 1981

CURRENT ANNUAL LEVEL: $9859

PROJECT DESCRIPTION

OBJECTIVES: To determine the prechronic toxicity of 1,2,3-trichlorpropane and to establish data relevant for a subsequent chronic study.

METHODS EMPLOYED: Both sexes of F344 rats and B6C3F1 mice were dosed with the chemical by gavage in corn oil in a 17-week subchronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: Both study reports were received from the laboratory in FY83. Work on this contract has been completed and it has been terminated. The Chemical Manager needs to evaluate the data to determine if further testing is warranted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1,2,3-Trichloropropane is an industrial solvent which is chemically similar to other short chain halogenated hydrocarbons that are known carcinogens.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 22180
NIH-NO1-ES-95655-02

TITLE: Carcinogen Bioassay of Acetonitrile

CONTRACTOR'S PROJECT DIRECTOR: William B. Coate, Ph.D.

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: August 31, 1981

CURRENT ANNUAL LEVEL: Zero

PROJECT DESCRIPTION

OBJECTIVES: To determine the prechronic toxicity of acetonitrile and to establish data relevant for a subquent chronic study.

METHODS EMPLOYED: Both sexes of F344 rats and B6C3F1 mice were exposed to acetonitrile by the inhalation route. Studies involved 6-hour exposures in 14-day repeated dose and 90-day subchronic studies.

MAJOR FINDINGS AND PROPOSED COURSE: The 90-day subchronic study was reported and the contract was terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Acetonitrile is a high production chemical and is used in the production of numerous pharmaceutical and agricultural chemicals. Thus, the potential for exposure to a large segment of the population is possible.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 22180
NO1-ES-95655-03

TITLE: Carcinogen Bioassay of C.I. Acid Red 114, 3,3'-Dimethylbenzidine, C.I. Direct Blue 15 and 3,3'-Dimethoxybenzidine

CONTRACTOR'S PROJECT DIRECTOR: Borge M. Ulland, D.V.M.

PROJECT OFFICER (NIEHS): Joseph E. Tomaszewski, Ph.D.

DATE CONTRACT INITIATED: September 30, 1981

CURRENT ANNUAL LEVEL: $750,000 (Est.)

PROJECT DESCRIPTION

OBJECTIVES: To determine the toxicity and carcinogenicity of 3,3'dimethylbenzidine (DMB) and 3,3'dimethoxybenzidine (DMOB) vs. a DMB-derived and a DMOB-derived dye as part of a benzidine class study.

METHODS EMPLOYED: All four chemicals have been administered to both sexes of F344 rats and B6C3F1 mice in their drinking water.

MAJOR FINDINGS AND PROPOSED COURSE: The 90-day subchronic studies of C.I. Acid Red 114 and DMB were completed in FY83. Reports on the subchronic studies of all four compounds were delivered and doses were set for the chronic studies. All four chronic studies are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
These studies are part of a program that is designed to provide the scientific information required for making regulatory decisions about the benzidine and benzidine congener-based dyes, serve as a model for future chemical class studies, and conserve research resources.

TITLE: Master Agreement for the Conduct of Toxicity and Carcinogenicity Studies in Laboratory Animals

Contractor	Contractor's Project Director	Date Contract Initiated
Battelle Memorial Institute 505 King Avenue Columbus, OH 43201 (NO1-ES-2-8002)	Dr. A. Peters	May 31, 1982
Battelle Pacific-Northwest Labs. P.O. Box 999 Richland, WA 99352 (NO1-ES-2-8003)	Dr. Wlm. Clark	July 6, 1982
Bioassay Systems Corporation 225 Wildwood Avenue Woburn, MA 01801 (NO1-ES-2-8004)	Dr. Indu Muni	May 31, 1982
EGG Mason Research Institute 57 Union Street Worcester, MA 01608 (NO1-ES-2-8010)	Dr. H. Lilja	June 1, 1982
Food and Drug Research Laboratories, Inc. Route 17C P.O. Box 107 Waverly, NY 14822 (NO1-ES-2-8005)	Dr. Peter Becci	June 1, 1982
Gulf South Research Institute P.O. Box 1177 New Iberia, LA 70561 (NO1-ES-2-8019)	Mr. Ralph Wheeler	June 1, 1982
Hazleton Laboratories America 9200 Leesburg Pike Vienna, VA 22180 (NO1-ES-2-8023)	Dr. B. Ulland	May 31, 1982
Hazleton-Raltech, Inc. P.O. Box 7545 3301 Kinsman Blvd. Madison, WI 53707 (NO1-ES-2-8006)	Karen Mackenzie	June 1, 1982

Contractor	Contractor's Project Director	Date Contract Initiated
Illinios Institute of Technology Research Institute 10 West 35th Street Chicago, IL 60616 (NO1-ES-2-8007)	Barry Levine	June 1, 1982
International Research and Development Corporation 500 N. Main Street Mattawan, MI 49071 (NO1-ES-2-8008)	Dr. D.C. Jessup	June 1, 1982
Litton Bionetics 5516 Nicholson Lane Kensington, MD 20795 (NO1-ES-2-8009)	Dr. A. Manus	June 1, 1982
Microbiological Associates 5221 River Road Bethesda, MD 20816 (NO1-ES-2-8011)	Dr. M. Dinowitz	May 31, 1982
Midwest Research Institute 425 Volker Blvd. Kansas City, MO 54110 (NO1-ES-2-8012)	Dr. Shellenberger	May 31, 1982
Papanicolaou Cancer Research Institute 1155 Northwest 14th Street P.O. Box 016188 Miami, FL 33101 (NO1-ES-2-8020)	Dr. Altman	June 31, 1982
Southern Research Institute 2000 Ninth Avenue South P.O. Box 3307-A Birmingham, AL 35205 (NO1-ES-2-8013)	Dr. J.D. Prejean	June 1982
Springborn Institute for Research 553 N. Broadway Spencerville, OH 45887 (NO1-ES-2-8014)	Dr. Fulff	May 31, 1982

Contractor	Contractor's Project Director	Date Contract Initiated
SRI International 333 Ravenswood Avenue Menlo Park, CA 94025 (NO1-ES-2-8015)	Dr. W. Davis	May 31, 1982
Toxicity Research Laboratories, Ltd. 510 W. Nackley Avenue Muskegon, MI 49444 (NO1-ES-2-8016)	Dr. E.C. Tompkins	June 1982
ToxiGenics, Inc. 1800 East Pershing Road Decatur IL 62526 (NO1-ES-2-8017)	Dr. Taylor	May 31, 1982

PROJECT OFFICER (NIEHS): Mina Lee Vernon, Ph.D., Head Collaborative Services, Program Operations Branch, TRTP

CURRENT ANNUAL LEVEL: $0.00

PROJECT DESCRIPTION

OBJECTIVES:

To provide a pool of laboratories capable of performing required toxicity and/or carcinogenicity tests under specified conditions.

METHODS EMPLOYED:

Acute, 14-Day Repeated Dose, 90-Day Repeated Dose, and/or 104-Week Repeated Dose tests may be done on laboratory animals using such routes of administration as gavage, dosed feed, dosed water, inhalation, and/or skin paint. In addition to clinical observations, other specific toxicologic parameters may be determined such as hematology, clinical chemistry, neurological effects, and/or reproductive effects. A general statement of work stipulating overall Program requirements is supplemented by specific protocols designed to get the maximum information needed for each chemical on test under any given task order award.

MAJOR FINDINGS AND PROPOSED COURSE:

Twelve task order awards have been made since October 1, 1982 for toxicity and/or carcinogenicity tests on twelve chemicals. The major findings are detailed elsewhere under specific task order awards that have been made under the Master Agreement contracts.

In the past, Master Agreement awards have been made for a two year period. Future awards will be for a five year period.

SIGNIFICANCE TO BIOMEDICAL RESERCH AND THE PROGRAM OF THE INSTITUTE:

Testing of chemicals for toxicological and carcinogenic potential is a specific goal of the National Toxicology Program. The Master Agreement contract awards provide a pool of laboratories capable of performing the required tests under specific conditions thus facilitating, through task order competition, the initiation of tests on chemicals which are of paramount importance because of population exposure.

PROGRAM RESOURCES BRANCH

ANNUAL REPORT OF THE PROGRAM RESOURCES BRANCH
TOXICOLOGY RESEARCH AND TESTING PROGRAM
Summary Statement

The Program Resources Branch provides the essential aspects for the Toxicology and Carcinogenesis studies conducted by the National Toxicology Program, namely Analytical Chemistry, Chemical Health and Safety and Animal Care. Branch staff procure, analyze and mointor chemicals for these studies as well as supply disease-free rodents to serve as the test system. The chemical health and safety group monitors each testing laboratory and each study within a test facility for those factors which may adversely effect 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 17 chemicals for the general in vivo toxiciology studies. In addition, 34 chemicals were obtained and analyzed for other programs within the National Toxicology Program such as teratology studies, immunotoxicology studies, reproductive toxicology studies, and continuous breeding experiments. Services were provided for the analysis of bulk chemical, chemical in test vehicles, methods development for quality assurance, including purity, stability (both bulk chemical and chemical/vehicle mixtures) and concentration determinations, chemical residue analysis of body tissues and fluids and special handling for residual and reprocured chemicals. In addition, tissue and body fluid residual analyses were developed and performed to enhance data from toxicity experiments of four chemicals. Eleven hundred and fifty aliquots of test chemicals were dispensed and shipped to labs under contract to the cellular and genetic toxicology program.

The Branch maintains repositories for chemical compounds which are currently under study or which have completed testing in the various NTP programs including the carcinogenesis testing program and the genetic toxicology program. Over 1400 individual chemicals from these testing programs are stored and maintained in these repositories.

The chemical health and safety aspects of the NTP are also the responsibility of the Program Resources Branch. Frequently, special safety requirements are needed because of a particular chemical's properties or the unique needs of the specific toxicology experiment. Input from chemical health and safety for NTP studies were provided for initial experimental design to insure special requirements are met and to provide chemical specific health and safety guidelines. Involvement continued through initial laboratory evaluation, follow-up site visits, program reviews, report monitoring, recommended changes in procedures, facilities design, etc., as well as response to problem emergency situations and concerns with eventual waste disposal and record archiving. Baseline safety evaluations of each facility contracted to the NTP were updated to ensure human health and the integrity of each study are not jeopardized. In addition, chemical monitoring and industrial hygiene surveys for specific chemicals; periodic review of training needs at the contract labs; and formulations of standards, guidelines and safety plans were completed in FY 1983.

The Program Resources Branch provides genetically defined rodents of known

microbial status for the various NTP testing and research programs. Two rodent production facilities are currently under contract to provide F-344 rats and B6C3F1 mice at weekly intervals for th NTP toxicology and carcinogenesis testing programs. For FY 1983, 32,000 mice of each sex and 32,000 rats of each sex were provided by these contracts. Rodents are also supplied by the Division of Cancer Treatment of the National Cancer Institute at its Frederick Cancer Research Facility (FCRC). Disease monitoring continued to be the priority for animal resources to maintain the quality of the animals supplied to NTP supported studies. One diagnostic laboratory under contract to NTP and three other diagnostic laboratories under contract to DCT,NCI are conducting disease monitoring of the rodent production facilities at monthly intervals. The sentinel animal program established in FY 1980 to monitor the prechronic and chronic toxicity and carcinogenicity studies continues to serve as the monitoring program for viral infections in each animal room at the toxicology testing laboratories under contract to the NTP. The genetic integrity of the production and test rodents is currently monitoried by histocompatability (skin graft) or biochemical (isozyme electrophoresis) means under two contracts to DCT,NCI. An additional contract to provide a genetic monitoring resource for the NTP was established in FY 1983.

RESEARCH TRIANGLE INSTITUTE, RESEARCH TRIANGLE PARK, NORTH CAROLINA
(NO1-ES-38044)

TITLE: Genetic Monitoring of Inbred Rodents

CONTRACTOR'S PROJECT DIRECTOR: R. Wayne Hendren, Ph.D.

PROJECT OFFICER (TRTP): Ghanta N. Rao, D.V.M., Ph.D., Animal Resources, Program Resources Branch, TRTP

DATE CONTRACT INITIATED: March 2, 1983

CURRENT ANNUAL LEVEL: $63,941

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to provide genetic monitoring resource for biochemical genetic monitoring of each generation of inbred stock at all production facilities producing F344/N rats and B6C3F1 hybrid mice to the toxicology research and testing program of the NTP as well as the animals supplied to the testing facilities.

METHODS EMPLOYED: Up to 15 designated loci will be monitored for each strain or hybrid by electrophoresis of erythrocyte lysates, kidney homogenates and serum proteins. Twenty parent generation animals of B6C3F1 hybrid mice or 20 hybrid mice and 5 F344 rats from each of the foundation colonies, production facilities and one of the testing facilities will be evaluated for genetic integrity at monthly intervals. In addition frozen tissues from 50 B6C3F1 hybrid mice per month will be subjected to isoenzyme analysis by electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Technical proficiency of the contractor in distinguishing the various allelic variants and hybrid types of rodent tissues is being evaluated. After successful demonstration of the technical proficiency; parent generations of B6C3F1 hybrid mice, the hybrid mice, F344 rats and frozen tissues from the foundation colonies, production facilities, and testing laboratories will be evaluated for genetic homogeneity at monthly intervals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Fischer 344/N rats and B6C3F1 hybrid mice are the selected experimental animal species for evaluation of chemicals for toxicologic and carcinogenic properties under TRTP/NTP. The genetic integrity of the test animals is essential for developing reliable and accurate research data in animal experiments. Constant monitoring for biochemical genetic variants of rederived as well as production stock and test animals will ensure that data from NTP animal studies will be collected from genetically homogeneous rats and mice. Genetic homogeneity of test animals will be essential for program wide comparison of the background incidence of tumors and lesions between testing laboratories, between chemicals and within the same laboratory over time.

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 (TRTP): Douglas B. Walters, Ph.D., Head, Chemical Health and Safety, Program Resources Branch, TRTP

DATE CONTRACT INITIATED: September 28, 1981

CURRENT ANNUAL LEVEL: $587,951

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for up to 5000 compounds which are to be screened for toxicity testing in the Toxicology Research and Testing 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.

MAJOR FINDINGS AND PROPOSED COURSE: Approximately 900 aliquots have been shipped for FY 1983 testing. Currently, inventory at the repository is approximately 1,000 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 to be tested by the closed cup method.

An apparatus for determining the permeation of glove materials by TRTP test chemicals has been constructed and validation of the method by ASTM standards is completed. This work provides valuable information to help enable TRTP 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 Cellular and Genetic Toxicology (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).

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 Chemistry, 54, 1866-1871(1982).

McKinney, J.D., Moore, L., Prokopetz, A., and Walters, D.B.: Validated Extraction and Cleanup Procedures for Polychlorinated Biphenyls and 2,2(4-chlorophenyl)-1,1-dichloroethene in Human Body Fluids and Infant Formula, Analytical Chemistry (In press).

Trammell, R.L., Keith, L.H., and Prokopetz, A.T.: Practical Aspects of Packaging and Shipping Test Chemicals for Research in Chemical Health and Safety for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

A.D. LITTLE, INC, - CAMBRIDGE, MASSACHUSETTS
(NO1-ES-05673)

TITLE: Health and Safety Services Support for the Carcinogenesis Testing Program

CONTRACTOR'S PROJECT DIRECTOR: R. Scott Stricoff

PROJECT OFFICER (TRTP): Douglas B. Walters, Ph.D., Head, Chemical Health and Safety, Program Resources Branch, TRTP

DATE CONTRACT INITIATED: September 30, 1980

CURRENT ANNUAL LEVEL: $329,372

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to assist the Toxicology Research and Testing Program in the evaluation of health and safety practices of the TRTP 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 design of sampling strategies.

METHODS EMPLOYED: The Contractor furnishes services, qualified personnel, material, equipment and facilities as needed to evaluate, survey and assist the TRTP 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 TRTP test materials.

Development and evaluation of alternate work practices or engineering controls in alleviating potentially hazardous situations encountered in TRTP facilities.

Inspection of laboratory facilities for qualitative evaluation of the health and safety program.

Performance of detailed Health and Safety Surveys of TRTP facilities.

Preparation of chemical specific Health and Safety documents for use by TRTP contract personnel.

Response to special situations requiring rapid action because of potentially hazardous conditions.

Evaluation of Incinerator Design for proper disposal of various TRTP toxicity testing chemicals.

MAJOR FINDINGS AND PROPOSED COURSE: Coordination and planning meetings have occured and include the health and safety evaluation of: all TRTP contract laboratories; procedures used for disposal by incineration of surplus TRT

chemicals; medical monitoring programs applicable to TRTP Toxicology testing laboratories; the health and safety programs; procedures and facilities of current toxicology laboratories via participation and assistance in the routine monitoring and inspection program: practices and equipment used for personal protection; development of training needs and materials; study of materials handling procedures by tracers; evaluation of personnel safety in the design of tissue trimming and dosing stations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Toxicology Research and Testing 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 concerns for Toxicity Testing in Chemical Health and Safety 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 Chemical Health and Safety 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 Chemical Health and Safety for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Prokopetz, A.T. Baillargeon, W.S., Prescott, E.M., Walters, D.B. and Stricoff, R. S.: Preparation of Chemical Health and Safety Documents in Chemical Health and Safety 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 Chemical Health and Safety for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Walters, D.B., Jameson, C.W.: Chemical Health and Safety for Toxicity Testing. Ann Arbor Science, Ann Arbor, Michigan (In press).

Hoyle, E.R., Stricoff, R.S. and Walters, D.B.: Laboratory Hood Performance in Toxicity Testing Laboratories in Chemical Health and Safety for Toxicity Testing. Ann Arbor Science, Ann Arbor Michigan (In press).

RADIAN CORPORATION - AUSTIN, TEXAS
(NO1-ES-95649)

TITLE: National Toxicology Program Chemical Repository

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Acting Chief, Program Resources a Branch, TRTP

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $173,750

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for the chemicals which are studied by the 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, crossed 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 478 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 250 aliquots of chemicals have been shipped in FY 83. Also, approximately 200 aliquots of chemicals have been transferred to the NIEHS Chemical Repository for Mutagenicity Screening. In addition, bulk quantities of 78 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 detemine 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 maintenance 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-ES-95615)

TITLE: Chemical Servies Support for the National Toxicology Program

CONTRACTOR's PROJECT DIRECTOR: K.M. Stelting, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Acting Chief, Program Resources Branch, TRTP

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 chemistry 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 dosage mixtures. 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 83, 17 chemicals were procured and analyzed for carcinogenesis testing. In addition, eight 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 or synthesizing and analyzing 19 new chemicals for study and providing routine analytical chemistry services for the contract laboratories of this program. Support for other NTP Programs, including immunology, chemical disposition and the in vivo rat liver model was also accomplished with the procurement and/or analysis of seven new chemicals. Work was also completed on routine dose mixing and analysis as well as tissue and body fluid residue analysis for nine chemicals being studied by various members of the intramural TRTP staff. Future plans include continued support of the above mentioned activities as well as support of any new NTP initiatives.

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 object 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 toxicity studies. Without this activity, no substantive animal or in vitro testing could occur. The precise definition of the chemical nature of test compounds is one of the cornerstones in an effort to increase the accuracy and reliability of data obtained in toxicological research.

Title: Rodent Production Contracts

PROJECT OFFICER (TRTP/NTP): Ghanta N. Rao, D.V.M., Ph.D., Animal Resources, Program Resources Branch, TRTP

DATE CONTRACT INITIATED: September 30, 1979

CHARLES RIVER BREEDING LABORATORIES, STONERIDGE, NY NO1-ES-95617
CONTRACTOR'S PRINCIPAL INVESTIGATOR: Mr. Stephen P. Cail
CURRENT ANNUAL LEVEL: $318,586

SIMONSEN LABORATORIES, GILROY, CA NO1-ES-95643
CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. James D. Russell
CURRENT ANNUAL LEVEL: $334,258

FREDERICK CANCER RESEARCH FACILITY, FREDERICK, MD
PROJECT DIRECTOR: Dr. Joseph G. Mayo
CURRENT ANNUAL LEVEL: $275,000

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to produce disease-free, genetically homogeneous and microbiologically defined F-344 rats and B6C3F1 hybrid mice for the NTP toxicology research and testing programs.

METHODS EMPLOYED: Genetically defined inbred pedigree stock was obtained from the NIH repository and shipped to each breeding facility. Offspring from matings between these animals were cesarean derived, maintained in isolotors, and associated with defined microflora. These animals are being used as inbred foundation stock for each breeding facility. Offspring from the foundation stock are used to supply production colonies from which contractually specified number of each species are produced weekly. Rats and mice are being shipped to contract toxicology testing facilities one week after weaning. Pedigreed production breeder stocks are being replaced every 30 weeks from the foundation colonies maintained in isolators.

MAJOR FINDINGS AND PROPOSED COURSE: The F-344 rat and the B6C3F1 hybrid mouse serve as the primary animal test system for the NTP toxicology research and testing programs. For FY 1983, 40,000 mice of each sex and 40,000 rats of each sex were contracted for production for use in the testing program. However, due to virus contamination and excess production than required for the program, the production contract NO1-ES--95617 with Charles River Breeding Laboratories at the production facility in Stone Ridge, NY was terminated on April 14, 1983 for the convenience of the goverment. The actual production for FY 1983 will be 32,000 mice of each sex and 32,000 rats of each sex. This decrease in production will not delay starting of toxicity studies of any chemical for more than two weeks due to shortage of test animals. The animals produced from the remaining production facilities continue to be free of infectious diseases. Genetic monitoring has become an important aspect of the surveillance program. Each generation of inbred stock at all production facilities is being monitored for genetic integrity as well as diseases.

Simonsen laboratories successfully installed an innovative room size flexible film isolator for production of disease free F-344 rats and B6C3F1 hybrid mice. This facility in FY 1983 is producing 200 animals of each sex of each species per week in a microbiologically defined environment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The Toxicology Research and Testing Program (TRTP) under the National Toxicology Program (NTP) organizes and conducts a comprehensive interagency testing and research program focused on determining potential human health hazards due to exposure to chemicals. The toxicologic evaluation of chemicals is generally conducted through a sequence of experiments that involve acute, subacute, subchronic and chronic exposure of laboratory animals to chemical substances. The chronic rodent bioassay is the current preferred procedure for determining the carcinogenic potential of chemicals. Rats, mice and other small laboratory animals are appropriate species for evaluating toxicologic and carcinogenic properties of chemicals. Fischer 344/N rats and B6C3F1 hybrid mice are the selected experimental animal species for evaluation of chemicals for toxicologic and carcinogenic properties under NTP. AT least two centralized production facilities of F344 rats and B6C3F1 hybrid mice with homogeneous genetic properties and defined microbial status will insure an adequate and continous supply of defined quality animals for the Toxicology Research and Testing Program.

SYSTEMIC TOXICOLOGY BRANCH

SYSTEMIC TOXICOLOGY BRANCH
Summary Statement

Prediction of the potential for chemicals to adversely affect human health is best accomplished through extrapolation from toxicological data collected in laboratory animals. Programs within the Systemic Toxicology Branch, in combination with those of other branches in the Toxicology Research and Testing Program (TRTP), are designed to collect data to help characterize the toxicological profile of chemicals and also to collect data which help improve the methods for toxicological evaluation as well as better understand the mechanisms of toxicity of selected chemicals.

The Systemic Toxicology Branch consists of five sections: Biochemical Toxicology, Chemical Disposition, Fertility and Reproduction, Immunotoxicology, and Inhalation Toxicology. Each section is summarized below; for more details and specific accomplishments, consult the individual presentations on the following pages.

Biochemical Toxicology: Structure-activity studies of chemicals are done to ascertain the mechanisms of action at the molecular and biochemical level. Major projects involve the identification and characterization of chemically-induced alterations in cytochrome P-450(s). These enzymes are responsible for metabolism of exogenous chemicals. Studies are in progress to examine changes in the genetic control of various subspecies of cytochrome P-450 in the rat after treatment with PCBs, TCDD, and other environmental chemicals.

Chemical Disposition: Investigates the absorption, distribution, metabolism, and excretion of a range of chemicals to provide information which will be useful for the design and interpretation of studies of chemical toxicity and carcinogenicity. Studies of chemical disposition are 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 they contribute to our basic understanding of chemical toxicity and help 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 chemicals such as furan, 2,3-dibromopropanol, halogenated dibenzo furans, bromo-naphthalenes, p-phenylenediamine, and 2,6-dichloro-p-phenylenediamine, and allyl isothiocyanate. Studies on these chemicals include evaluation of the effect of species, sex, dose, and route of exposure on disposition. Studies on the effect of age on metabolism of exogenous chemicals continue to be conducted. In addition, disposition studies were conducted on a large number of chemicals which are being evaluated for chronic toxicity and carcinogenicity in the NTP carcinogenesis evaluation program.

Fertility and Reproduction: Studies were conducted in in-house laboratories to assess the effect of various chemicals on reproduction and fertility in males and females. These studies included such known reproductive toxins as glycol ethers, dimethyl methyl phosphonate, DBCP, and phthalate esters. Through contract mechanisms, studies continue which are designed to assess methods to detect adverse effects of chemicals on reproductive function or capacity. These include a program to assess continuous breeding trials as a means of assessing the effective chemicals on fertility as well as evaluations on animals in subchronic toxicity studies to assess sperm morphology and sperm counts as well as vaginal cytology in rats.

Immunotoxicology: Studies in this section continue to evaluate the influence of selected environmental chemicals on the immune system of animals, to relate alterations in immunological functions with both general toxicity and organ-specific toxicity, and to relate changes in immunological function with alterations in host resistance. In addition, work continues to refine and validate a panel of immune and host resistance procedures which can be used to better define immunotoxicity and correlate changes in immune function with altered host resistance. Data from these studies help characterize the toxicologic profile of chemicals, including those being evaluated for other toxicologic endpoints elsewhere in the NTP.

Inhalation Toxicology: The program of the Inhalation Toxicology section includes the design and execution of studies of compounds to which toxicologically significant exposure could be expected to be primarily by the inhalation route. Research is focused on manifestations of toxicity at the levels of tissues, organs, and organ systems. The in-house program is integrated with that of Northrop Services, Inc., an on-site contractor with responsibility for conducting research on inhalation toxicology in an exposure facility that is separate from the in-house facility.

Specific studies in progress include an evaluation of the effects from simultaneous exposure to morpholene and nitrogen dioxide. Also, studies are in progress to assess the usefulness of the Strain A mouse as a model for inhalation carcinogenesis In addition, studies are in progress to evaluate the problems of hypothyroidism and the acceleration of atherosclerosis that accompanies long-term low level exposure to carbon disulfide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21003-03 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Halogenated Dibenzofurans

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

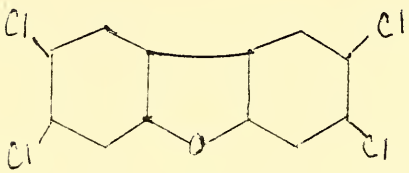
Halogenated dibenzofurans are found worldwide as environmental pollutants. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the degree and position of halogenation. This work has established that 2,3,7,8-tetrachlorodibenzofuran (TCDF), an extremely toxic isomer, is excreted only after metabolism and toxicity is inversely related to metabolic capability. The concept of a threshold body burden for toxicity is currently being tested. The distribution to the fetus will be examined after maternal exposure. The role of body composition on the disposition of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), the most toxic man-made compound known, is being examined in congenic mouse strains which are sensitive or resistant to TCDD toxicity.

Principal Investigator and All Other Personnel Engaged on the Project:

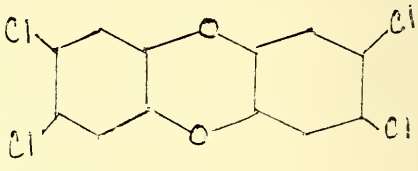
Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
H. B. Matthews	Research Chemist	TRTP	NIEHS
Yiannakis M. Ioannou	Staff Fellow	TRTP	NIEHS
Hans Weber	Visiting Fellow	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has used radioactively labeled compounds to quantitate absorption, distribution, metabolism and excretion, TCDF, and TCDD. TCDF was labeled with ^{14}C; TCDD was labeled with ^{3}H. The disposition of TCDF has been studied after repeated exposure in guinea pigs. The disposition of TCDD has been studied in 4 strains of mice: C57BL/6, Ah^B/AH^D (responsive); C57BL/6, Ah^D/Ah^D/non-responsive): DBA/2, Ah^B/Ah^D(responsive); DBA/2, Ah^D/Ah^D (non-responsive). The distribution to the fetus will be determined by treating pregnant C57BL/6 mice at various times during gestation and following the appearance and localization of ^{14}C-TCDF in the fetuses.



TCDF



TCDD

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:

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.

Studies with DBA (non-responsive) and C57BL (non-responsive) mice with TCDF suggested that body composition could modulate the topic response observed in these mice, ie., "fat" mice (DBA) were less sensitive to the toxic actions of compounds like TCDD than were the "lean" mice (C57BL). The strains differ at the Ah locus, which may mediate the toxic response to TCDD and congeners. However, these strains differ at many other loci. To test the role of body composition vs. the Ah locus, congenic mice were obtained from Dr. A. Poland (University of Wisconsin). The distribution and excretion of ^{3}H-TCDD is being examined in two groups of mice, DBAs, both responsive and non-responsive, and C57BL, both responsive and non-responsive.

The distribution of TCDF to the fetus is also being examined using pregnant C57BL/6J mice, treated on days 10 or 11 of gestation and sacrificed from 24-96 hours later. TCDF has recently been shown to be a potent teratogen, ED_{50} $\sim$ 30 µg/kg on day 10 (H. Weber, in preparation).

PUBLICATIONS

King, F.G., Dedrick, R. L., Collins, J. M., Matthews, H. B. and Birnbaum, L. S.: Physicological Model for the Pharmacokinetics of 2,3,7,8-Tetrachlorodibenzofuran in Several Species. Toxicol. Appl. Pharmacol. 67:390-400, 1983.

Matthews, H. B. and Birnbaum, L. S.: Factors affecting the disposition and persistence of halogenated furans and dioxins. In. Tucker, R. E., Young, Al.L., and Gray, A. P. (Eds): Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, NY, Plenum Publish. Corp., 1983, pp. 463-475.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21004-03 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Senescent Changes in Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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, small intestine, brain, lymphoid tissues. Enzyme systems such as glucuronyl transferase, β-glucuronidase, and those involved in intermediary metabolism and immune responses will be investigated. Altered distribution and excretion of chemicals in aging animals is being studied in order to elucidate the basis for age-related changes in pharmacological responses. Age-related alterations in gastrointestinal absorption are also being studied.

Principal Investigator and All Other Personnel Engaged on the Project:

Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Michael Dieter	Research Physiologist	TRTP	NIEHS
William C. Eastin	Research Physiologist	TRTP	NIEHS

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 interim colony of retired breeder F344 male rats has been maintained at NIEHS. Animals available to us thus range in age from 1 through 36 months of age.

For studies of age-related changes, old animals (>24 months) will be compared to young adult animals (2-6 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 intestine. No changes in the specific activity of either enzyme was observed in extracts from lung or intestine between animals of 3,6,12,18, and 32 months of age. In both liver and kidney there was a decline in activity of glucuronyl transferase between 3 and 6 mos of age with no further age-related decrease. β-Glucuronidase activity increased in kidney homogenates and microsomes as the rats aged. The enzymatic changes are being correlated with pathological alterations as well.

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, suggesting a slower rate of metabolism. Increased metabolite/parent ratios in the tissues suggested a slower elimination of metabolites in the old rats. Since the effects of aging seem to be more pronounced for 245 than for 236, senescence may differentially affect the disposition of more persistent PCBs. Age-related changes in body composition seem to modulate the dispositional properties.

Age-related changes in absorption of xenobiotics from the small intestine are being investigated using the technique of in situ animal perfusion. Initially, both passive and active transport will be compared in young (3 mo.) and old (24 mo.) rats using model compounds. Chemicals of environmental interest will be used to see if age-related effects on absorption occur.

Another aspect being initiated involves the in vivo study of age-related changes in metabolism of xenobiotics. Rats of different ages will be treated with radioactively labeled xenobiotics and the metabolites excreted in the urine and bile analyzed for both quantitative and qualitative changes.

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.

PUBLICATIONS

Birnbaum, L. S.: Changes in the disposition of two hexachlorobiphenyls in senescent rats. In. Kitani, K. (Ed.), Liver and Aging-1982: Liver and Drugs, Amsterdam, Elsevier Biomedical Press, 1982, pp. 99-113.

Birnbaum, L.S.: Distribution and Excretion of 2,36,2',3',6'- and 2,4,5,2',4',5'-hexachlorobiphenyl in senescent rats. Toxicol Appl. Pharmacol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21009-03 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reproductive Effects in Males Exposed to Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James C. Lamb, IV Research Biologist STB TRTP/NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch
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:
2.5	1.25	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 glycol ethers, dimethyl methyl phosphonate, 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 testicular morphology, 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.

Principal Investigator and All Other Personnel Engaged on the Project:

James C. Lamb, IV	Research Biologist	STB	TRTP/NIEHS
R.E. Chapin	Staff Fellow	STB	TRTP/NIEHS
J.A. Moore	Deputy Director, NTP		NIEHS
E.E. McConnell	Chief	CPB	TRTP/NIEHS
J.K. Dunnick	Biologist	CTEB	TRTP/NIEHS

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. Fertility is evaluated by various mating trial protocols.

MAJOR FINDINGS AND PROPOSED COURSE: Studies have been conducted on the effects of DBCP, DMMP, glycol ethers and phthalate esters 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 chemical's toxic mechanisms. Such information will allow us to develop more predictive test systems in this field.

PUBLICATIONS

Kluwe, W.M., Gupta, B.N., and Lamb, J.C., IV: Comparative effects of 1,2-dibromo-3-chloropropane and its metabolites epichlorohydrin, alphachlorohydrin and oxalic acid in the urogenital tract. Toxicol. App. Pharmacol., in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21024-02 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Environmental Chemicals on Drug-Metabolizing Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. A. Goldstein Pharmacologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Biochemical Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this study are to examine changes in genetic control of subspecies of cytochrome P-450 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 immunoprecipitation and in vitro translation of messenger RNAs for various P-450's after treatment with PCBs and other environmental chemicals. Changes in translation and isozyme content will be examined with time and dose of inducer. Antibodies are being raised to additional forms of rat liver P450 to be used as probes for quantitating changes due to environmental chemicals and other factors such as hormones and aging. We will attempt to clone DNA complementary to some of the rat P450s to use to analyze gene structure and changes in protein and mRNA synthesis after exposure to various chemicals. The goal of this project is to better understand the changes occurring at the genetic level after exposure to environmental chemicals.

Principal Investigator and All Other Personnel Engaged on the Project:			
J. A. Goldstein	Pharmacologist	TRTP	NIEHS
J. Hardwick	Staff Fellow	TRTP	NIEHS
P. Linko	Chemist	TRTP	NIEHS
R. Weaver	Biological Lab Tech	TRTP	NIEHS
M. Caveness	Q-Appointment	TRTP	NIEHS

PROJECT DESCRIPTION

MAJOR FINDINGS: 1) Mutagenesis experiments were completed with three isozymes of P-450. The two 3-methylcholanthrene (MC) (P-448_{55} and $P448_{52}$) inducible isozymes were the most active in metabolizing aromatic amines such as 2-aminofluorene and Trp-P-2 (a pyrolysis product of tryptophan) to mutagenic products. Only one isozyme ($P448_{55}$) metabolized benzo[a]pyrene to mutagenic products. All three isozymes metabolized aflatoxin. Nitrosamines were not activated by any of the isozymes.

2) Using a sensitive RIA and a western blotting procedure to SDS-polyacrylamide gels combined with immunostaining, we have quantitated amounts of two isozymes in various extrahepatic tissues after 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The response to TCDD is tissue dependent. TCDD induces two isozymes dramatically in liver. One of these isozymes ($P448_{52}$) is a minor constitutive form. However, only one isozyme was induced in extrahepatic tissues.

3) Two additional isozymes have been isolated from rat liver and antibodies to these forms are being produced in rabbits.

4) Work on the metabolism of 2-acetylaminofluorene (AAF) is almost completed, including assessment of the contribution of two MC-inducible isozymes to the metabolism of AAF in microsomes of control, MC and polychlorinated biphenyl induced microsomes. The two MC inducible isozymes are very active in hydroxylation of AAF and both produce some N-hydroxylation.

PROPOSED COURSE: Experiments involving immunoquantitation of isozymes and in vitro translation of the 3-MC inducible messages in the rat are in progress and will be examined to determine whether the messages are expressed co-ordinately. Purification of additional rat cytochromes including constitutive cytochromes from control rats is in progress. DNA complementary to these cytochromes will be cloned to obtain probes to study control and modulation of genetic function. The importance of some of these monooxygenases to the metabolism and activation of various substrates such as acetaminophen, aminofluorene and steroids will be examined in our laboratory or through collaborative studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The P-450 system is the principle monooxygenase system which catalyzes foreign chemicals as well as a number of endogenous compounds in genetic control of these enzymes. Probes developed in the rat will be particularly useful because of the wide use of this species in toxicology and carcinogenicity studies. These probes will also be useful for studying developmental and hormonal effects on regulation of this system. Probes may be used across species because of the similarity of many of these enzymes in various species.

PUBLICATIONS

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., 131-139, 1982

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., 31, 1607-1613, 1982.

Robertson, I.G.G., Zeiger, E., and Goldstein, J. A.: Specificity of rat liver cytochrome P-450 isozymes in the mutagenic activation of benzo[a]pyrene, aromatic amines and aflaxtoxin B_1. Carcinogenesis 4: 93-96, 1983.

Luster, M.I., Lawson, L.D., Linko, P., and Goldstein, J. A.: Immunochemical evidence for two 3-methylcholanthrene inducible forms of cytochrome P-448 in rat liver microsomes using a double-antibody radioimmunoassay procedure. Mol. Pharmacol. 23: 252-257 (1982).

Sundheimer, D. W., Caveness, M., and Goldstein, J. A.: Catalytic differences between 3-Methylcholanthrene inducible forms of rat liver cytochrome P450. Arch. Biochem. Biophys., In press, 1983.

Goldstein, J. A. and Linko, P.: Differential induction of two 2,3,7,8-tetra-chlorodibenzo-p-dioxin inducible forms of cytochrome P-450 in extrahepatic versus hepatic tissues. Mol. Pharm. In press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES21025-02 STB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Disposition of Santonox

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
PI: Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

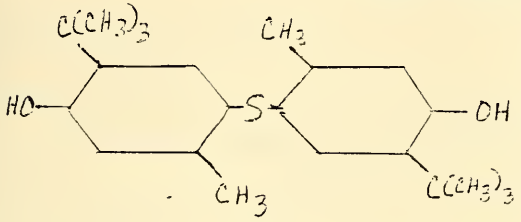
SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Santonox causes severe gastroenteritis after oral exposure. After iv administration, the compound was rapidly metabolized in the liver and excreted via the bile into the feces. About 10% of the dose persists in the liver, skin and adipose tissue, suggesting the possibility of accumulation upon chronic exposure.

Principal Investigator and All Other Personnel Engaged on the Project:

Linda S. Birnbaum	Reserch Microbiologist	TRTP	NIEHS
H. B. Matthews	Research Chemist	TRTP	NIEHS
W. C. Eastin	Research Physiologist	TRTP	NIEHS
Richard Smith	Visiting Fellow	TRTP	NIEHS

PROJECT DESCRIPTION



Santonox

METHODS EMPLOYED: ^{14}C-labeled Santonox was used in order to study the chemical chemical disposition of this antioxidant in Fischer 344 rats. Absorption from the gastrointestinal tract was determined at 3 doses, the highest being 1/10th of the oral LD_{50}. Rate of absorption by the small intestine was measured using an *in situ* luminal perfusion assay. The distribution after an iv dose was 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.

MAJOR FINDINGS AND PROPOSED COURSE: Absorption of Santonox was incomplete after oral exposure. The rate of absorption was decreased with increasing dose due to enhanced retention in the stomach, which also resulted in severe irritation and ulceration. This irritant effect was not due to the vehicle or the volume used, but was a reflection of the actual amount of Santonox delivered by a bolus gavage dose. Once reaching the small intestine, absorption was proportional to the dose. Treatment by the iv route resulted in rapid distribution from the blood to the rest of the body with the principal depots at early times being liver, muscle, skin and adipose tissue. Santonox was rapidly metabolized by the liver and excreted primarily via the bile into the feces. Some of the parent compound tended to persist in the liver, skin, and adipose tissue. This might allow accumulation to occur upon chronic exposure.

Glucuronide conjugates appear to be the major metabolites of Santonox, based on enzymatic digestion studies. The parent compound, as well as the metabolites, are also base-sensitive. Identification and characterization of the metabolites of Santonox is currently in progress. The techniques to be used include NMR and GC-MS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21026-02 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Hexabromonaphthalene

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

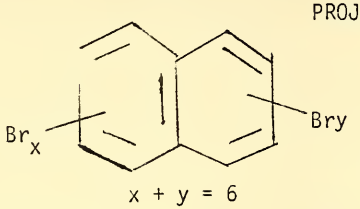
SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bromonapthalenes have no known industrial use or application, but have 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 a mixture of 2-hexabromonapthalenes (HBNs), previously identified as a single isomer, 1,2,3,4,6,7-HBN. The compound is incompletely absorbed after an oral dose. After iv treatment over 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. These disposition results led to proof of the presence of two isomers by high resolution NMR, present in a ratio of 60:40 which have been tentatively identified as 1,2,3,4,6,7- and 2,3,4,5,6,7-HBN.

Principal Investigator and All Other Personnel Engaged on the Project:

Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
James D. McKinney	Research Chemist	LEC	NIEHS

PROJECT DESCRIPTION



HBN

x + y = 6

METHODS EMPLOYED: This work has used ^{14}C-labeled compound to quantitate the chemical disposition of 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. Resolution of the compound into two isomers was accomplished using high resolution NMR.

MAJOR FINDINGS AND PROPOSED COURSE: 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. Toxic insult occurred to the liver initially, but by 35 days the livers had recovered. The discovery by NMR that the HBN was in fact a mixture of two isomers was compatible with the speculation that the major isomer (~60% of the total) was toxic, but rapidly metabolized and excreted while the minor isomer (40%) was persistent and not metabolized. The major isomer was tentatively identified as 1,2,3,4,5,7-HBN and the minor as 2,3,4,5,6,7-HBN. So far, attempts to resolve these isomers by HPLC or GC have not been successful, but work is continuing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21027-02 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Allyl Isothiocyanate: Comparative Disposition in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Yiannakis M. Ioannou Senior Staff Fellow TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The absorption, distribution, metabolism and excretion of ^{14}C-labeled allyl isothiocyanate (AITC) was studied in male and female rats and mice. AITC was cleared from all rat and mouse tissues so that within 24 hours after administration less than 5% of the total dose was retained in the tissues. Clearance of AITC-derived radioactivity by each species was primarily in urine (70-80%) and as CO_2 in exhaled air (10-15%) with lesser amounts in feces (2-5%). Over 98% of AITC was metabolized by rats and mice to 5 or 6 metabolites respectively. These results demonstrated significant species related differences in AITC metabolism and sex related differences in urine volume excreted. These variations result in exposure of male rat urinary bladders to significantly higher concentrations of an AITC metabolite for longer periods of time and may account for similar variations in sensitivity to bladder toxicity by AITC.

Principal Investigator and All Other Personnel Engaged on the Project:
Yiannakis M. Ioannou Senior Staff Fellow TRTP NIEHS

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 and $B6C3F_1$ mice. 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 of all major tissues, oxidation of samples to $^{14}CO_2$ and quantitation of radioactivity by liquid scintillation counting. Major tissues, urine, feces and bile were extracted by different solvents and the metabolites present were analyzed 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 of both rats and mice, distributed to all tissues examined and rapidly excreted in urine in the form of several metabolites. Minor quantities are excreted in feces (2-5%). The half-life for clearance from most tissues was less than 6 hr and less than 10% 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 of rats and mice was primarily in the form of metabolites with only traces of parent compound present at all time points examined. The metabolites excreted in the urine of male and female rats were qualitatively similar but showed small quantitative variations. Metabolites in male mouse urine were different quantitatively from those present in the female mouse urine. Urinary bladders from AITC-treated male rats contained over 6-18 fold higher concentrations of AITC at early time points (15 min to 6 hr) than did those from female rats. At the same time, female rats (AITC-treated or untreated) excreted twice as much urine (volume per kg body weight) than did the male rats.

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. Neither female F344 rats nor either sex of $B6C3F_1$ mice exhibited any carcinogenic effects as a result of chronic administration of AITC. Since AITC is present in foods, the potential for human exposure is very high. The results of the present study offer a possible explanation as to the species and sex specificity of AITC toxicity and should prove useful to risk/benefit assessments regarding human health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21029-02 STB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Influence of Kepone on Female Reproduction

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
James C. Lamb, IV Research Biologist STB TRTP/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 *(Use standard unreduced type. Do not exceed the space provided.)*

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. Pituitary cell responses in vitro are also evaluated. 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.

Principal Investigator and All Other Personnel Engaged on the Project:

James C. Lamb, IV	Research Biologist	STB	TRTP/NIEHS
J.A. Moore	Deputy Director, NTP		NIEHS
E.E. McConnell	Chief, CPB		TRTP/NIEHS
K.S. Korach	Research Endocrinologist	LRDT	NIEHS
J.S. Hong	Pharmacologist	LBNT	NIEHS

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 chlorodecone 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.

MAJOR FINDINGS AND PROPOSED COURSE: Studies in which Kepone was added to the diet showed that even though 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.

SIGNIFICANCE OF 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

Ali, S.F., Hong, J.S., Lamb, J.C., IV, Moore, J.A., and Bondy, S.C.: Subchronic dietary exposure to rats to chlordecone (Kepone®) modifies levels of hypothalamic β-endorphin. Neurotoxicology, 3:119-124, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21036-01 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of Benzo(f)quinoline

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

PI: Linda S. Birnbaum Research Microbiologist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

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

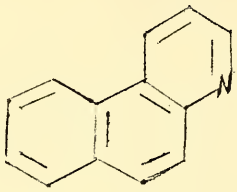
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Benzo(f)quinoline has been recommended for study in the NTP as a mutagenic air pollutant and as a representative of nitrogen-containing aromatic heterocyclic compounds. It is present in various crude oils. Preliminary studies indicate that it may have carcinogenic potential. 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 for toxicity studies, but a better understanding of the mechanism of toxicity of this compound.

Principal Investigator and All Other Personnel Engaged on the Project:
Linda S. Birnbaum Research Microbiologist TRTP NIEHS

PROJECT DESCRIPTION



Benzo-f-quinoline

METHODS EMPLOYED: ^{14}C-labeled benzo(f)quinoline (BQ) is being used to study the disposition of this chemical in male Fischer 344 rats after acute oral and iv exposure. Analyses of radioactivity were facilitated by the use of a biological materials oxidizer and liquid scintillation counting. Tissue extraction with organic solvents was followed by high performance liquid chromatography in order to resolve parent compound from metabolites.

MAJOR FINDINGS AND PROPOSED COURSE: BQ is completely absorbed from the gut. It initially concentrates in the liver. Much of the compound is rapidly metabolized in the liver and excreted in approximately equal amounts via the bile into feces or via the kidneys into the urine. A portion of the total dose is distributed to the fat and skin, from which it returns to the liver. By 72 hrs after treatment, less than 2% of the dose is retained in the body. The majority of the tissue radioactivity is due to unmetabolized BQ, while most of that excreted represents oxidation products of the parent compound. The metabolites of BQ will be further characterized and the possibility of covalent binding to tissue macromolecules investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Benzo(f)quinoline has been selected for carcinogenicity testing because of its mutagenic activity, its structural similarity to quinoline, a known carcinogen, and the potential for human exposure. No studies on its metabolism have been reported. These studies of its disposition will allow a better design to be developed for toxicity testing as well as provide more information on structure/activity relationships.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21038-01 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical Metabolism and Disposition

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

H. B. Matthews Research Chemist TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of chemical metabolism and disposition are designed to provide both applied knowledge in support of chronic toxicity tests conducted by the National Toxicology Program and basic knowledge of those chemical structure and property relationships which determine toxicity. Studies of furan indicate that the disposition of this compound is highly dependent upon both route of administration and the dose administered. Furan is eliminated primarily in the exhaled air. Metabolism of furan involves the formation of reactive intermediates which covalently bind to both protein and nucleic acids. Studies of dermal absorption of 2,3-dibromopropanol indicate that it is absorbed through the skin, but most of the dose applied to skin volatilizes and may be inhaled or lost to the atmosphere.

Principal Investigator and All Other Personnel Engaged on the Project:
H. B. Matthews Research Chemist TRTP NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The absorption, distribution, metabolism and excretion of chemicals are studied following iv, oral or dermal administration of the chemical of interest to adult male rats and/or other species as the objectives of the studies dictate. Absorption and distribution of chemicals to tissues and clearance from tissues into excretia are quantitated by utilizing ^{14}C-labeled compounds serial sacrifice and analytical techniques which facilitate quantitation of radioactivity in biological media. Equipment used includes metabolism cages designed to permit separate collection of urine, feces and exhaled air, biological material oxidizers to convert organic compounds to CO_2 and liquid scintillation to quantitate ^{14}C in biological samples and CO_2. 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: 1) The disposition of furan has been studied in the male rat following oral, iv and ip administration of doses ranging from 1 to 100 mg/kg. Metabolism of furan has been studied both in vivo and in vitro. Results of these studies indicate that the disposition is dependent upon both the route of administration and the dose administered. The higher the dose the larger the percent of dose eliminated as the parent compound in exhaled air. Elimination in exhaled air is also highly dependent upon route of administration and is greatest following iv administration. Furan administration results in depletion of glutathione from liver and covalent binding of furan metabolites to liver proteins and nucleic acids. Covalent binding in liver is greatest following oral administration. Furan is metabolized to reactive intermediates by hepatic mixed-function oxidases requiring NADPH and the metabolites are conjugated with glutathione.

2) The disposition of 2,3-dibromopropanol has been studied following dermal and iv administration. Studies of disposition following dermal administration were facilitated by a new technique which was developed to permit quantitation of evaporation from the skin as well as absorption while at the same time preventing an unrestrained rat from licking or rubbing the site of application. Results of this study indicates that following dermal administration most of the dose volatilizes from the surface and a lesser amount is absorbed, metabolized and rapidly excreted. Following iv administration 2,3-dibromopropanol is rapidly metabolized and excreted.

PROPOSED COURSE: Each of the above studies will be completed and the results reported to the appropriate chemical manager and in the literature. Additional compounds will be studied as the need and opportunity arises. Additional assistance, in the form of a Visiting Fellow, will be recruited to increase the number and diversity of projects which can be undertaken.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chemicals vary widely in their properties and toxicities to higher animals. However, the health threat posed to humans by various chemicals can not be accurately assessed prior to careful study. Therefore, the goal of laboratory research on chemical toxicity is to extrapolate those results to humans. Extrapolations of laboratory observations of chemical toxicities to humans are most relevant when based on knowledge of chemical structure-activity relationships. Therefore, the goal of the present work is to gain a greater understanding of those chemcal structure-activity relationships which impinge upon chemical metabolism, disposition, persistence and toxicity in higher animals. The significance of this work to biomedical research and the program of the Institute is that results of these studies help explain how various chemical toxicities are mediated and what steps can be taken to avoid or minimize chemical toxicities and thus provide a safer environment.

PUBLICATIONS

Nomeir, A. A. and Matthews, H. B.: Metabolism and disposition of the flame retardant tris (2,3-dibromopropyl)phosphate in the rat. Toxicol. Appl. Pharmacol. 67: 357-369, 1983.

Matthews, H. B. and Birnbaum, L. S.: Factors affecting the disposition and persistence of halogenated furans and dioxins. In: Tucker, R.E., Yound A.L. and Gray, A.P. (Eds.). Human and Environmental Risks of Chlorinated Dioxins and Related Compounds. Plenum Press, New York. pp. 463-475, 1983.

Chopade, H. and Matthews, H. B.: Disposition and metabolism of 4-chloronitro-aniline in the male F-344 rat. J. Toxicol. Environ. Hlth. 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.

Matthews, H. B., Surles, J. R., Carver, J. G., and Anderson, M. W.: Halogenated biphenyl transport by blood components. Fund. Appl. Toxicol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21040-01 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

2,6-Dichloro-p-phenylenediamine: Comparative Disposition in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Yiannakis M. Ioannou Senior Staff Fellow STB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the absorption, distribution, metabolism and excretion of ^{14}C-labeled 2,6-dichloro-p-phenylenediamine (DCPDA) in the male and female rats and mice indicate that this compound is readily absorbed, rapidly metabolized and excreted mainly in the urine in the form of several metabolites. All radioactivity excreted in the feces represented parent compound. DCPDA does not appear to accumulate in any tissue and the whole body half-life was less than 1 day in both rats and mice. Rats and mice excreted the same major metabolite in urine. However, this metabolite is at least 2-fold higher in mice than in rats. It is possible that the higher toxicity of DCPDA to mice is due to the presence of higher quantities of this major metabolite.

Principal Investigator and All Other Personnel Engaged on the Project:			
Yiannakis M. Ioannou	Senior Staff Fellow	TRTP	NIEHS
H. B. Matthews	Research Chemist	TRTP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has utilized ^{14}C-labeled 2,6-dichloro-p-phenylene-diamine (DCPDA) in order to quantitate absorption, distribution accumulation, metabolism and excretion of DCPDA in male an female F344 rats and B6C3F$_1$ mice. Major tissues, urine and feces were extracted by suitable solvents and the metabolites present were analyzed and quantitated by HPLC. Kinetic parameters were based on the disposition data and were calculated by computer. The possible binding of DCPDA and DCPDA metabolites to DNA and other macromolecules was also investigated.

MAJOR FINDINGS: Following oral administration, DCPDA is absorbed from the gastrointestinal tract, distributed to all tissues assayed, metabolized to a great extent and excreted in the urine or feces in the form of several metabolites. DCPDA did not appear to bioaccumulate in any particular tissue Only minor amounts of DCPDA or metabolites (<5%) were present in the tissues 3 days after administration in both male and female rats and mice. Male rats excreted an additional metabolite in urine which was not present in female rats or either sex of mice. Female rats excreted twice as much parent compound in urine as males and over 10-fold higher than female mice. Both rats and mice excreted the same major metabolite in urine but the amount excreted by mice was at least 2-fold greater than that excreted by rats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: DCPDA was recently shown to be a positive carcinogen to male and female mice causing hepatocellular adenomas and carcinomas but a negative carcinogen in male and famale rats. The present study indicates a possible mechanism(s) for this species specific DCPDA toxicity may be variations in DCPDA metabolism. These results should be useful in evaluating and interpreting the findings of the NTP chronic toxicity study and facilitate extrapolation of these studies to humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21041-01 STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disposition of p-Phenylenediamine in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Yiannakis M. Ioannou Senior Staff Fellow TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

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 *(Use standard unreduced type. Do not exceed the space provided.)*

The absorption, distribution, metabolism and excretion of p-phenylenediamine (PDA) has been studied in male and female rats and mice. PDA is readily absorbed, distributed to all tissues, metabolized and excreted mainly in urine in the form of several metabolites in both rats and mice. The rate of clearance from the tissues is fairly rapid and the whole body half-life is less than 12 hours. Only muscle retained significant quantities (4-7%) of PDA-derived radioactivity 24 hours after an iv dose. Quantitative and qualitative differences were observed between rat and mouse urine metabolites.

Principal Investigator and All Other Personnel Engaged on the Project:

Yiannakis M. Ioannou	Senior Staff Fellow	TRTP	NIEHS
H. B. Matthews	Research Chemist	TRRP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has utilized ^{14}C-labeled p-phenylenediamine (PDA) in order to quantitate absorption, distribution, accumulation, metabolism and excretion of PDA in male and female F344 rats and B6C3F$_1$ mice. Major tissues, urine and feces were extracted by suitable solvents and the metabolites present were analyzed and quantitated by HPLC. Possible binding of PDA and PDA metabolites to DNA was investigated using hydroxylapatite column chromatography for DNA purification.

MAJOR FINDINGS: Following oral or iv administration PDA was rapidly distributed to all tissues assayed, readily metabolized and excreted mainly in the urine (60-75% of dose) and in feces (15-25% of dose). There were no significant differences in absorption, distribution and excretion between male and female rats and mice. Quantitative differences in metabolism were observed between the sexes of rats and mice. However, both quantitative and qualitative differences in metabolism were observed between the two species. There was no evidence of binding of PDA or any of its metabolites to liver DNA.

SIGNFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PDA is the major component of many oxidation hair dyes and along with other aromatic amines has been implicated in increasing cancer risk among dye manufacturing industry workers. Although PDA was not found to be carcinogenic in F344 rats and B6C3F$_1$ mice, the present study was carried out in order to investigate different disposition parameters and compare them to those of other aromatic amines (i.e. 2,6-dichloro-p-phenylenediamine) which were shown to be carcinogenic. These results might prove useful in structure-activity relationships of aromatic amines and in the choice of the safest types of chemicals to be used in hair dyes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES21042-01 STB

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tumorigenic Potential of Nitrogen Dioxide by Inhalation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

E.W. Van Stee	Veterinary Officer	STB	TRTP/NIEHS

COOPERATING UNITS *(if any)*
Northrop Services, Incorporated

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Mice were exposed to approximately 20 ppm of nitrogen dioxide (NO_2) for 5-6 hr, or 1 g/kg of body weight of morpholine by gavage, or both. Treatments were repeated daily for 5 consecutive days. N-nitrosomorpholine (NMOR) was found in whole carcasses (16 to 146 ng/mouse) in all animals that had been exposed to both NO_2 and to morpholine, but not in tissues from animals that had been exposed to either chemical alone. Approximately one-third of the NMOR was found in the gastrointestinal tract. The coadministration of 2 g/kg of sodium ascorbate or 1 g/kg of alpha-tocopheryl acetate had no effect on the amount of NMOR that was found in any tissue. Approximately one-third of the total amount of NMOR that was found in the body was found in the stomach. Another possible product of the interaction of NO_2 and morpholine, N-nitromorpholine, was not detected in any tissue. We concluded that the repeated, concurrent exposure of mice to NO_2 by inhalation and to morpholine by gavage resulted in the in vivo formation of significant quantities of NMOR. The biological significance of the observation remains unknown.

Principal Investigator and All Other Personnel Engaged on the Project:

E.W. Van Stee	Veterinary Officer	STB	TRTP/NIEHS
Richard A. Sloane	Biologist	STB	TRTP/NIEHS
Jane Ellen Simmons	Graduate Student	STB	TRTP/NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Mice were exposed daily for 5 consecutive days to 20 ppm of NO2 or plain air for 6 hr, with or without concurrent exposure to 1 g/kg of morpholine by gavage. Trachea-lung, stomach and gastric contents, and whole mice were analyzed for the presence of N-nitrosomorpholine and N-nitromorpholine by gas chromatography (thermal energy analysis).

2. Female Strain A/J mice from 6-8 wk old were randomly assigned to 12 groups of 20 each. Three groups were injected IP with urethane in 0.9% NaCl at each of the following dosages: 0, 250, 500 and 1000 mg/kg of body weight. Groups representing each dosage were killed at 4, 5, and 6 months post-injection, respectively. Lungs were fixed with Tellyesniczky's solution and examined 24 hr later by 3 technicians, working independently, for the presence of pulmonary adenomas. Representative tissues were saved for histopathological examination.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Lung concentrations of morpholine averaged 23.5 +/- 27.8 micrograms/g of wet tissue weight during the first 15 wk of exposure (n=6). Pulmonary adenomas were found from 509 through 673 days postexposure. The rates were controls 3.8% (1/26); NO2 3.8% (1/26); morpholine 7.1% (2/28) and NO2 + morpholine 21.2% (7/33). Several statistical procedures were employed in the evaluation of these data. The results of these analyses were similar (p ranged from 0.04 to 0.08) and suggested that the combination of NO2 and morpholine increased the probability of the occurrence of lung adenomas in the mouse. One nasal adenocarcinoma has been found so far in one rat from the group treated with NO2 + morpholine. Most, but not all, of the rats from all 4 treatment groups have been examined. This line of work is being continued on NIEHS contract NO1-ES-79-0009 (Northrop Services, Inc.).

2. The results of the study of the in vivo formation of N-nitrosomorpholine and N-nitromorpholine indicate that significant amounts of N-nitrosomorpholine, but not of N-nitromorpholine, are formed in mice exposed concurrently to morpholine by gavage and to NO2 by inhalation. Total body burdens and distribution of N-nitrosomorpholine were not affected by the co-administration of ascorbic acid or alpha-tocopheryl acetate.

3. The concurrent exposure of Strain A/J mice to 10 ppm NO2 and 0.1% morpholine for 6 months did not increase the formation of pulmonary adenomas above background.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: These studies have been designed to determine if exposure to NO2 can, in special circumstances, pose an environmental health hazard beyond that of a respiratory tract irritant. The ubiquity and inescapability of this by-product of industrialization mandates that we recognize the full meaning of its presence to the

public health. Experiments in this series have been designed to determine the mechanism of the formation of putative mediators of oncogenesis formed by the in vivo interaction of NO2 with nitrosatable amines. We have demonstrated that the concurrent exposure of mice to NO2 and nitrosatable amine results in the in vivo formation of measurable quantities of N-nitrosamine. The biological significance of this observation remains to be determined.

PUBLICATIONS

Van Stee, E.W., Sloane, R.A., Simmons, J.E., Brunnemann, K.D. (1983): In vivo formation of N-nitrosomorpholine in CD-1 mice exposed by inhalation to nitrogen dioxide and by gavage to morpholine. J. Natl Cancer Inst 70(2): 375-379.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES21043-01 STB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Chemical-Induced Immunotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)
Richard W. Pfeifer Senior Staff Fellow STB NIEHS

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:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The on-going objectives of this project are to develop predictive hypotheses about chemical-induced immunotoxicity. This can be achieved with a knowledge of the distribution and metabolism of a compound in conjunction with an understanding of the molecular biology inherent in in vitro assays of immune cell function. We are in the process of developing three immunoregulatory circuit models based on recognized (cancer chemotherapeutic agents, benzene) and postulated (estrogens) mechanisms of chemical/immunological interaction. The models include: (1) lectin-induced lymphocyte agglutination (early event) in conjunction with blastogenesis (late event); (2) lymphokine-induced macrophage agglutination (early event) in conjunction with cytostasis (late event) and analysis of lymphokine production by lectin-stimulated lymphocytes using these assays; and (3) lymphocyte and macrophage-mediated cytostasis. This approach should potentially identify chemical metabolites which have "specific" effects on various components of host defense.

Principal Investigator and All Other Personnel Engaged on the Project:

Richard W. Pfeifer	Senior Staff Fellow	STB	NIEHS
Rachel M. Patterson	Biological Lab Technician	STB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mature blood cell components of the organism, including the immune system, are functionally responsive cell types that must be continually replenished by precursor cells cycling in various stages of differentiation. The plasma membrane, cytoskeletal apparatus (microtubules, microfilaments), and other transducing structures such as calmodulin, control the mobility and local density of specific receptors for growth factors and hormones in the immediate environment. These membrane events which occur during the initial minutes of cell-ligand interaction appear to determine the course taken by the cell: positive signal (proliferation, differentiation) versus a negative signal (suppression, tolerance). These controlling mechanisms are sensitive to chemical insult in many different cell types, however, the immune system is particularly vulnerable since its very existence depends on the integrity of the signalling apparatus for continued growth and differentiation.

We are in the process of developing three immunoregulatory circuit models which are based on the aforementioned premise as a basis for understanding immunotoxicity. The models analyze the effects of the metabolites of a parent compound on lymphocyte and macrophage activation and corresponding effector or cell functions and include:

1. lectin-induced lymphocyte agglutination and blastogenesis;
2. lymphokine-induced macrophage agglutination and cytostasis (and analysis of lymphokine production by lectin-stimulated lymphocytes using these assays); and
3. lymphocyte and macrophage-mediated cytostasis.

MAJOR FINDINGS AND PROPOSED COURSE: This is the first report regarding this project which has been in existence less than one year. Much has been accomplished during these initial months including:

1. planning a laboratory and ordering instrumentation and reagents suitable for investigation of cellular and molecular mechanisms of immunotoxicity;
2. hiring and training (ongoing) a laboratory technician in the necessary techniques applicable to this type of research;
3. developing the three proposed immunoregulatory circuit models (ongoing) for use in this laboratory. As these models are based on a mechanistic understanding of established and postulated modes of immunotoxicity, some are entirely new assays or represent significant modifications of already established methodology. Although the features of the models are such that they are optimally designed for *in vitro* analysis of mechanisms, there is no reason why some models could not be adapted to the systematic screening of chemicals for immunotoxicity.

(1) Model 1: Increased adherence and agglutinating properties of a variety of cell types including macrophages, lymphocytes and polymorphonuclear neutrophils are observed as a consequence of activation at the cell surface. Such changes occur very early on, often within minutes after the triggering event. In many models, for example, lectin-induced lymphocyte agglutination, controversy exists as to whether increased adherence properties are necessary or associated with effective cell activation.

Using a highly sensitive method to measure lectin (phytohemagglutinin, or PHA)-induced agglutination spectrophotometrically, we suggest that although increases in the agglutination of activated lymphocytes may not be correlated with blastogenesis at supraoptimal concentrations of PHA (those associated with the inhibitory limb of the blastogenic response curve), there is a correlation when optimal blastogenic concentrations of lectin are used. That alterations in membrane properties resulting in increased adherence are associated with the positive signal for blastogenesis is suggested by the observation that the effects of sulfhydryl (SH)-reactive catechol estrogen metabolites (2-OH estrone and 2-OCH_3 estrone) as well as non-reactive metabolites (estrone and 16α-OH estrone) on PHA-induced blastogenesis is accompanied by identical effects, whether enhancing or inhibitory, on the agglutination response of activated cells occurring within minutes. This correlative activity occurs at every concentration of compound and lectin, and suggests a role for estrogens in modulating lymphocyte activation at the cell surface (Modulation of lectin-stimulated lymphocyte agglutination and mitogenesis by estrogen metabolites: R.W. Pfeifer and R.M. Patterson, in preparation).

Catechol estrogen metabolites (2-OH estrone) inhibit lectin-induced lymphocyte agglutination ($5x10^{-5}$ - 10^{-6}M). Spleen, thymus and non-adherent cells of the peritoneal cavity (predominately lymphocytes) are equally susceptible. As reported previously for the polyhydroxy metabolites of benzene (including catechol) in conjunction with PHA-induced blastogenesis, the modulation is biphasic (at μM concentrations of metabolite, enhancement of responses occurs at supraoptimal lectin concentrations) and SH-dependent. Although inhibition of agglutination can be observed visually in these experiments, spectrophotometric analysis indicates unequivocally that enhancement of blastogenesis is correlated with enhancement of agglutination observed within minutes of lectin addition to cells.

In the absence of a catechol structure, the metabolites (estrone) alter membrane properties ($5x10^{-5}$ - $5x10^{-6}$M) such that responses to PHA are enhanced. Agglutination indices from individual experiments demonstrate that this comitogenic activity varies inversely with the effectiveness with which PHA is able to induce a positive activating response. Therefore, the degree of inhibition of cell surface receptor mobility (in response to different concentrations of PHA) determines the alteration of cell response by estrogen

metabolites. At lower concentrations of estrone (μM), suppression of responses is observed which appears unrelated to lectin concentration. We are in the process of investigating what role, if any, specific receptor binding plays in the mediation of any of these effects. It is interesting that the catechol estrogen metabolite 2-OCH_3 estrone retains SH-reactive properties (as for 2-OH estrone) while demonstrating comitogenic activity (as for estrone); the properties can be separated by analysis at different lectin concentrations.

(2) Model 2: Thymectomy protects against some of the estrogen-induced immunotoxicity in vivo, particularly the suppression of bone marrow precursor cells observed at lower doses. Also, macrophage activation is observed after exposure to estrogens. Indirect estrogen effects on lymphakine production by T cells and/or macrophage responsiveness to these mediators may account for some of these suppressive effects.

In the mouse, resident peritoneal cells (PECs) are a composite of macrophages (55-75%) and non-adherent cells (mostly lymphocytes). We have found that separation of PECs on plastic results in an adherent cell population (macrophages) which does not respond to PHA whereas the non-adherent cell population does so quite effectively. Therefore, PHA-induced agglutination is specific for lymphocytes. Alternatively, the response of PECs to concanavalin A-stimulated spleen cell supernatents containing lymphokine activity (macrophage activating factor or MAF) can be entirely attributed to the adherent cell population. If purified splenic lymphocytes are incubated with such supernatents, absorbances are decreased below baseline (incubations in the presence of "mock" MAF). Although we are uncertain as to the meaning yet, preliminary experiments suggest that an additional toxicity is demonstrated by 17B estradiol, 2-OH estrone and 2-OCH_3 estrone in the MAF-induced agglutination response of PECs relative to the ability of the compounds to inhibit PHA-induced lymphocyte agglutination.

(3) Model 3: We have been successful in developing a lymphocyte-mediated cytostasis assay which measures the cytolytic potential of T cells sensitized to allogeneic tumor cells in vivo. Use of high energy gamma isotopes, technical considerations related to the spontaneous release of labeled targets and total releaseable counts, and the harvesting of individual supernatants are circumvented. At the present time, there is no reason to suspect that the methodology would not be applicable to non-sensitized lymphocyte killing (NK) or MAF-activated macrophage-mediated cytostasis. Catechol and non-reactive estrogen metabolites modulate T cell cytostasis in an identical fashion to that described for PHA-induced lymphocyte agglutination and blastogenesis. This suggests that the estrogens act at the cell surface to modulate the early events of effector cell - target cell interactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Much data collected over the last ten years suggests that many environmental chemicals and potentially therapeutic agents, in the absence of significant toxicity to other organ systems, modulate immunological host defense mechanisms. Although it is known that various classes of compounds may be "specific" for cell-mediated versus humoral immunity, or may have effects on macrophage function, almost nothing is understood about the chemical/biological interactions that result in immune dysfunctions. Because of the complexity of cell-cell interactions in an immunologic reaction, the incomplete understanding of immunologic activation mechanisms, and the wide variety of in vitro assay systems in use to measure immune derangement, it is impossible to tell how such specific effects are related to differences in target cell susceptibility.

In view of attempts of clinicians to enhance selective components of host defense in particular disease states (reduction of side effects of cancer chemotherapy) while reducing them in others (treatment of autoimmune diseases), this lack of understanding of basic mechanisms appears extraordinary. With the mandate to set regulatory policy and safeguard the public health, toxicology is now faced with a similar dilemma with regard to hundreds of suspect chemicals. Much is known about the molecular events associated with the activation, differentiation and effector cell function of lymphocytes and macrophages. Therefore, these cells are not only important targets in immunotoxic response, but they represent valuable tools for the study of agents which alter growth and differentiation. We propose that predictive hypotheses about chemical-induced immunotoxicity can be achieved with an appropriate understanding of the molecular biology of in vitro immunological models and a consideration of individual metabolites of the parent compound.

PUBLICATIONS

Irons, R.D. and Pfeifer, R.W.: Benzene-induced immunotoxicity: the lymphocyte as a tool for studying subcellular mechanisms of toxicity. In Proceedings of the Nato Advanced Studies Institute of Immunotoxicology, in press (1983).*

Luster, M.I., Boorman, G.A., Hayes, H.T., Dean, J.H., Hong, L., Pfeifer, R., Korach, K.S. and Rhodes, L.: Environmental estrogens and their effect on immune responses. In Proceedings of the Nato Advanced Studies Institute of Immunotoxicology, in press (1983).

Pfeifer, R.W. and Irons, R.D.: Alteration of lymphocyte function by quinones through a sulfhydryl-dependent disruption of microtubule assembly. Int. J. Immunopharmacol., in press (1983).*

Pfeifer, R.W. and Irons, R.D.: Quinones and sulfhydryl-dependent immunotoxicity. In Proceedings of the 13th Conference on Environmental Toxicology, in press (1983).*

* Work performed at C.I.I.T. but mechanisms and methodology apply to this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30044-07 STB

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Toxicology of Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

E.W. Van Stee	Veterinary Officer	STB	TRTP/NIEHS

COOPERATING UNITS *(if any)*
Northrop Services, Incorporated

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Certain limitations on the flexibility of small animal inhalation exposure systems are overcome by the machine control and monitoring of the concentration of the gas or vapor of interest. Computer assistance of chamber operation allows the user to simulate time-varying concentration profiles accurately and repeatedly. We exposed rats to 7 different profiles in which the maximum concentration of carbon tetrachloride (CCl_4) was 1500 ppm and the concentration times-time (C x T) was 4500 ppm-hr. The purpose was to determine the effects of systematically varying the shape of the concentration profile on the expression of hepatotoxicity of a chemical about which much was already known. All of the exposures were conducted within a span of 6 hours. Examination of the severity of vacuolation and pattern of necrosis could be used to distinguish some of the exposure profiles from others. For example, vacuolation was less severe when 2 equal pulses were presented with an interval of 60 minutes, rather than 180-240 minutes. The indexes of necrosis varied in a more complex way and the differences among the profiles that accounted for the differences in the patterns of the histopathological changes were not immediately apparent. We concluded that the characteristic of a time-related variation in concentration is one of the determinants of the inhalation hepatotoxicity of CCl_4 and that the simple, time-weighted average concentration may not always fairly represent the best model for the study of problems in inhalation toxicology.

Principal Investigator and All Other Personnel Engaged on the Project:

E.W. Van Stee	Veterinary Officer	STB	TRTP/NIEHS
Richard A. Sloane	Biologist	STB	TRTP/NIEHS
Jane Ellen Simmons	Graduate Student	STB	TRTP/NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Male, CD rats were exposed in groups of 12, to 7 different exposure profiles of carbon tetrachloride (CCl_4) in which the maximum concentration was 1500 ppm. The exposures were conducted in a computer-assisted inhalation facility in dynamic flow-through chambers of 400 liters capacity. 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 (C x T) 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. 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 (CS_2), 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 wk. 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. Forty-eight male, NZW rabbits were divided into groups of three and exposed to all possible combinations of 0 or 2% dietary cholesterol, 0 or 300 ppm of CS_2, 0 or 0.1 mg/da of levothyroxine, and 0 or 25 mg/da of thiourea, respectively, for 12 wk. Changes in serum 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.

4. The effects of carbon disulfide exposure on hepatic lipid metabolism were studied in male Fischer F344 rats. Oxidative metabolism of carbon disulfide had been stimulated by the addition of phenobarbital, 0.1%, to the drinking water, beginning 5 days prior to the initiation of carbon disulfide exposure. Rats were exposed in groups of 3 or 4 to carbon disulfide by inhalation for 6 hours/day in dynamic flow-through chambers. The concentration of CS_2 in the chambers was measured continuously on samples drawn from the geometric center. Control rats were exposed to conditioned air. Rats were killed approximately 14 hours after the last carbon disulfide exposure, during the dark part of a 12 hour light-dark cycle. The rate of biosynthesis of cholesterol was estimated by determination of the rate of incorporation of 2-14C-acetate into cholesterol in liver slices from freshly killed rats.

5. Thirty female, strain A/J mice were exposed to 300 ppm of CS2 6 hr/da, 5 da/wk for 6 months. An equivalent group was exposed to air to serve as controls.

6. Seventy male and 70 female strain A/J mice were exposed to each of 0, 50, and 500 ppm of vinyl chloride 6 hr/da, 4 da/wk for 6 months.

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 dependence 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.

2. Serum T4, but not T3 or TSH, concentrations decreased with continuing exposure to carbon disulfide in rabbits, but not in rats. Rabbits were selected as a suitable model for the experiment described in paragraph 3.

3. The final report on the rabbit, CS2-cholesterol-T4-thiourea interaction study is in preparation.

4. The exposure of female, strain A/J mice to 300 ppm of carbon disulfide for 6 hr/da, 5 da/wk for 6 months did not result in a significantly accelerated rate of development of pulmonary adenomatosis in exposed animals as compared to controls.

5. Exposure of rats that had been pretreated with phenobarbital to 600 ppm carbon disulfide for 6 hr lowered cholesterol biosynthesis. Total hepatic cholesterol, esterified cholesterol plus unesterified cholesterol, was markedly increased. In rats exposed to phenobarbital plus 600 ppm carbon disulfide for either 2 or 3 days, the rate of cholesterol synthesis in the liver was decreased and the amount of cholesterol per gram of liver was increased. A concentration-response curve for each of these effects was constructed. Recovery following pretreatment with phenobarbital and a single exposure to 600 ppm carbon disulfide for 6 hours was measured.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 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. Furthermore, and of possibly greater near-term significance, these results underscore the importance of maintaining good control over the profiles to which

animals are exposed in any research or testing situation. All too often inhalation bioassays are performed by exposing animals to concentrations of chemicals that vary substantially above and below the target concentrations, with serious attention given only to the time-weighted average, ignoring the fluctuations. This practice can no longer be accepted.

PUBLICATIONS

Van Stee, E.W., Boorman, G.A., and Moorman, M.P. (1982): Time-varying concentration profile as a determinant of the inhalation toxicity of CCl4. J. Toxicol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-30106-04-STB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effects of Environmental Pollutants on the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

PI: Michael I. Luster Research Microbiologist STB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, National Toxicology Program, NIEHS

SECTION

Immunotoxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.75	2.0	2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ongoing objectives of the immunological-toxicology group include the following interrelated efforts: (1) to evaluate and examine the influence of selected environmental chemicals on the immune response including cellular changes associated with chemical interactions in lymphoreticular cells; (2) To relate alterations in immunological functions with both general toxicity as well as specific organ toxicity; (3) To relate changes in immunological functions with altered host resistance following challenge with either syngeneic tumor cells or infectious agents employing a defined panel of infectivity models and; (4) To refine and validate a panel of immune and host resistance procedures in order to better define immunotoxicity and correlate changes in immune function with altered host resistance. This approach should potentially allow for more accurate assessment of human health risk as well as determine no-effect levels for immunotoxic chemicals.

Principal Investigator and All Other Personnel Engaged on the Project:

Michael I. Luster	Research Microbiologist	STB	NIEHS
G.A. Boorman	Veterinary Medical Officer	CPB	NIEHS
A. Tucker	IPA; Medical College of Virginia	STB	NIEHS
K. Korach	Research Chemist	LPRT	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The assays employed 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 Panel for Defining Immune Alterations
Currently Being Employed at NIEHS

Parameter	Procedure Performed
Pathotoxicology	Hematology Profile-hemoglobin, red blood cell count, white blood cell count, differential
	Serum Chemistries - SGPT, BUN, Glucose, Total Protein, Albumin
	Weights-body, spleen, thymus, liver, kidney
	Histology-liver, thymus, adrenal, lung, kidney, heart, spleen
Host Resistance	Tumor Assays-tumor cell challenge TD10-20 and radiometric tumor mass
	Listeria monocytogenes LD_{10-20} challenge Streptococcus challenge LD_{10-20} challenge Plasmodium induced parasitemia
Marker Enumeration	Splenic B-cell, T-Cell and Lyt enumeration
Delayed Hypersensitivity	Radiometric assay with T-cell dependent antigen
Lymphocyte Proliferation	Mixed leukocyte culture
	Mitogens-PHA, Con A, LPS

Humoral Immunity	Antibody response to T-dependent (SRBC), T-independent (LPS), B1 (TNP-LPS) and B2 (TNP-Ficoll) antigens
Macrophage function[1]	Resident peritoneal cell numbers and differential
	Phagocytosis
	Ectoenzymes: 5'-nucleotidase, leucine amino peptidase and alkaline phosphatase
	Cytostasis of tumor target cells
Natural Killer Cell Activity	In vitro cytotoxicity using YAC-1 tumor cells
Bone Marrow Colony Forming Units	CFU-S-(hematopoietic stem cell proliferation)
	CFU-GM-(granulocyte/macrophage progenitor proliferation)
	59Fe-Incorporation into the bone marrow
	Cellularity

[1] Employs both resident peritoneal cells and MAF activated macrophages.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of chrysotile asbestos exposure on bone marrow and immune parameters were examined in mice at 6 and 12 months following a 3 day inhalation exposure. Ultrastructural examination revealed that the fibers were deposited primarily at alveolar duct bifurcations within the centriacinar region of the lung. Histological pulmonary changes were minimal but by 26 weeks early asbestosis characterized by clusters of macrophages and minimal fibrosis were present in the centriacinar region of the lung. Lymphoproliferative responses, antibody levels and number of plaque forming cells were not significantly altered in exposed mice. Pulmonary macrophages, but not peritoneal macrophages showed evidence of activation in the chrysotile exposed mice. The most striking change was the depression of bone marrow pluripotent stem cells (CFU-S) and marrow granulocyte macrophage progenitors (CFU-GM) which were decreased at all postexposure examinations. It is felt that the depression of bone marrow progenitors in asbestos exposed mice may have relevance to the leukopenia reported in workers with occupational history of asbestos exposure. These studies are concluding.

B6C3F1 mice were administered a total of 0, 20, 40, or 80 mg/kg of ochratoxin A intraperitoneally on alternate days over an 8 day period. There was a dramatic dose related decrease in thymic mass. Myelotoxicity was present as evidenced by bone marrow hypocellularity, decreased marrow pluripotent stem cells

(CFU-S), granulocyte-macrophage progenitors (CFU-GMs), and decreased ^{59}Fe uptake in marrows and spleens of exposed mice. There was also a depression of natural killer cell activity. Peritoneal macrophages from subcutaneously as well as intraperitoneally injected mice demonstrated increased phagocytic capacities and increased capacity to inhibit tumor cell growth. These alterations in bone marrow cells, macrophages, and NK cell activity occurred in mice at dosage levels that caused only minimal nephrotoxicity. This agent may operate, among other proposed mechanisms, by inhibition of protein synthesis in rapidly proliferating cell populations.

Preliminary studies are being conducted in an attempt to elucidate the mechanism of immune suppression by the inducers of aryl hydrocarbon hydroxylase (AHH). Because of its lack of carcinogenicity, we are presently investigating β-napthoflavone, a widely used AHH inducer. This compound produces an inhibition of the T-dependent humoral response seen with many other polycyclic aromatic hydrocarbons such as benzo(a)pyrene. One possible mechanism we are exploring is induction of acute phase proteins by AHH inducers. Serum from animals in the acute phase of the inflammatory response inhibits formation of antibody production in vitro in the Mishell-Dutton cultures. This provides a model for investigation of the AHH inducers.

Considerable effort has continued and will continue in immunotoxicology studies with compounds containing estrogenic activity. Mice exposed to steroidal and nonsteroidal estrogens including 17β-estradiol and diethylstilbestrol demonstrate immunotoxicity and altered host resistance. Non-estrogenic hormones including testosterone and progesterone failed to induce these effects. Immunotoxicity associated with estrogen exposure is regulated by a complex bimodal mechanism. One of these mechanisms is mediated through the thymus since surgical thymectomy abolished the ability of estrogens to suppress bone marrow proliferation, to activate macrophages and to alter host resistance using the infectivity models. Furthermore, supernatants of thymic epithelial cells cultured in the presence of estradiol were capable of inhibiting CFU-GM colony formation and activating macrophages. Specific immunotoxic events can also be disassociated chemically by estrogenic compounds such as zearalanol with poor uterotrophic activity, but reasonably effective binding affinity. Thus, many of these effects may depend upon relative receptor interaction of the compound or metabolites to appropriate target tissues which could then mediate hormonal effects on selective lymphoid tissues. Myelotoxicity is not mediated indirectly through the ovary or adrenal gland. That the initial events in estrogen-induced immunotoxicity may be mediated through a receptor mechanism was suggested by the ability of antiestrogens to induce antagonism when administered prior to estradiol and by the presence of estrogen binding components in lymphoreticular tissues including the thymus and bone marrow. Furthermore, immunotoxicity paralleled the degree of estrogenicity with non-estrogenic, genotoxic metabolites of diethylstilbestrol demonstrating very little myelotoxicity.

Preliminary studies have suggested that the pro-estrogen methoxychlor and its estrogenic metabolite didemethylated methoxychlor induce similar changes in selected immune response but require fairly high dosages because of their poor estrogen binding activity.

An abbreviated version of the assay panel had been 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. Immunotoxicity studies were completed for two chemicals, promethazine and methalilazine (H2-antagonists) in NTP contract laboratories. These compounds demonstrate minimum immunotoxicity primarily related to T-cell responses.

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) and more recently Penn (In: Current Problems in Cancer, Vol. 6, 1982) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was higher than in the general population. In another light epidemiological evidence has indicated that immunotoxic chemicals may act as predisposing agents in patients who develop Acquired Immunodeficiency Syndrome (e.g. Goedert et al., Lancet, 1982).

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 mechanisms and 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 NK 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. Furthermore, in order to accurately predict human health risk, no effect levels and structure-activity relationships, the mechanisms of immunotoxicity need to be more clearly defined.

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Luster, M.I., Boorman, G.A., Korach, K.S. Myelotoxicity resulting from exogenous estrogens: Evidence of bimodal mechanisms (Submitted).

Luster, M.I., Lawson, L.D., Linko, P., and Goldstein, J.A.: Immunochemical evidence for two 3-methylcholanthrene inducible forms of cytochrome P-448 in rat liver microsomes using a double-antibody radioimmunoassay procedure. Mol. Pharmacol. 23:252-257, 1983.

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ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE
Chicago, Illinois 60616
(NIH-N01-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 with respect to their ability to alter immune functions and host resistance to challenge with infectious agents or tumor cells. There are three 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 five 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 immunotoxic chemicals of environmental concern including diethylstilbestrol, cadmium chloride and dimethylnitrosamine. 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 three bacteria, influenza and Herpes simplex I as the two viruses, Trichinella spiralis as the parasite and the B16F10 as the transplantable tumor and more recently PYB6 tumor, Herpes simplex II and ^{35}S-Klebsiella pneumoniae, resistance models.

II. Immune Function Tests:

The following immune function assays are evaluated: (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; (6) Antibody response to a T cell-independent antigen; (7) Natural killer cell activity.

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 contractor has made substantial progress in all major tasks of the contract including: (1) development and a interlaboratory validation of sensitive and reproducible methods for evaluating host resistance to bacteria, viruses, animal parasites and transplantable tumors; (2) establishment and proficient demonstration of a standardized set of immunological tests; and (3) integration and validation of these test systems for detecting altered host resistance and immunological function using prototype chemicals with established immunotoxic profiles. The prototype chemicals employed during this year for these studies included a mild immunosuppressant ($CdCl_2$), a potent B-cell suppressant (dimethylnitrosamine), a mild B-cell immunosuppressant (ethyl carbamate), a potent B- and T-cell immunosuppressant (cyclophosphamide), and a macrophage activator (C. parvum). As a result of this systematic evaluation a sensitive and reproducible core of immunological assays and infectivity models to assess chemical-induced immunotoxicity have been selected (Table 1).

Table 1
Core Panel for Detecting Chemical-Induced Immunotoxicity

Parameter	Procedure Performed
Infectivity Models	• Streptococcus pneumoniae: LD_{20} + LD_{80} challenge • Listeria munocytogenes: LD_{20} + LD_{80} challenge • Herpes simplex virus II: LD_{20} + LD_{80} challenge • PYB6 Tumor Cell Challenge: TD_{20} + TD_{80} challenge • Plasmodium berghei: Percent parasitemia
Immunological Assays	• Natural killer cell activity • Delayed hypersensitivity response • Antibody response to T-DEP (SRBC), $T_{IND/B1}$ (TNP-LPS) and $T_{IND/B2}$ (TNP-Ficol) antigens • Mixed leukocyte cultures • Lymphocyte blastogenesis to mitogens
Toxicology Evaluation	• Body weight and selected organ weights • Hematology • Selected serum chemistries

Macrophage Functions	• Peritoneal cell numbers and differential • Phagocytosis • Ectoenzymes

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) and more recently Penn (In: Current Problems in Cancer, Vol. 6, 1982) 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. In another light, evidence has suggested that immunotoxic chemicals may act as predisposing agents in patients who develop Acquired Immunodeficiency Syndrome (AIDS) (e.g. Goedert et al., Lancet, 1982).

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 mechanisms and 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 Mφ 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.

Integration and validation of these test systems for detecting chemical-induced immunotoxicity using chemicals selected by NIEHS will take place in FY84.

MEDICAL COLLEGE OF VIRGINIA
Richmond, Virginia 23298
(NIH-NO1-ES-1-5001)

TITLE: Chemical-Induced Immunotoxicity

CONTRACTOR'S PROJECT DIRECTOR: Albert E. Munson, 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 with respect to their ability to alter immune functions and host resistance to challenge with infectious agents or tumor cells. There are three 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 five 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 immunotoxic chemicals of environmental concern including diethylstilbestrol, cadmium chloride and dimethylnitrosamine. 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. Also being examined are the PYB6 tumor cell challenge model, as well as the C. neoformans and N. Fowleri infectivity models.

II. Immune Function Tests:

The following immune parameters are evaluated: (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; (6) Natural killer cell activity; (7) Quantitation of T-independent antibody responses; (8) Serum complement levels.

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 to immune dysfunction.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor has made substantial progress in all major tasks of the contract including: (1) development and a interlaboratory validation of sensitive and reproducible methods for evaluating host resistance to bacteria, viruses, animal parasites and transplantable tumors; (2) establishment and proficient demonstration of a standardized set of immunological tests; and (3) integration and validation of these test systems for detecting altered host resistance and immunological function using prototype chemicals with established immunotoxic profiles. The prototype chemicals employed during this year for these studies included a mild immunosuppressant ($CdCl_2$), a potent B-cell suppressant (dimethylnitrosamine), a mild B-cell immunosuppressant (ethyl carbamate), a potent B- and T-cell immunosuppressant (cyclophosphamide), and a macrophage activator (C. parvum). As a result of this systematic evaluation a sensitive and reproducible core of immunological assays and infectivity models to assess chemical-induced immunotoxicity have been selected (Table 1).

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Macrophage Functions

- Peritoneal cell numbers and differential
- Phagocytosis
- Ectoenzymes

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) and more recently Penn (In: Current Problems in Cancer, Vol. 6, 1982) 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. In another light, evidence has suggested that immunotoxic chemicals may act as predisposing agents in patients who develop Acquired Immunodeficiency Syndrome (AIDS) (e.g. Goedert et al., Lancet, 1982).

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 mechanisms and 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 Mϕ 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.

Integration and validation of these test systems for detecting chemical-induced immunotoxicity using chemicals selected by NIEHS will take place in FY84.

LOVELACE INHALATION TOXICOLOGY RESEARCH INSTITUTE
Albuquerque, NM
(Interagency Agreement with the Department of Energy)
(22Y01-ES-20092)

TITLE: "Disposition of Inhaled Xenobiotics"

PROJECT DIRECTOR: John S. Dutcher, Ph.D.

PROJECT OFFICER (NIEHS): L. S. Birnbaum, Ph.D.

DATE CONTRACT INITIATED: September 30, 1982

CURRENT ANNUAL LEVEL: $325,685

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected volatile 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 excretion with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) Studies on the disposition of 2,3-dichloropropene (DCP), a chemical present with other chloropropene isomers in commercially used soil fumigants, have been initiated. Apparatus to generate vapors and expose rats to DCP were constructed and methods developed to analyze the compound. Recovery of radioactivity derived from ^{14}C-DCP was compared after oral and ip exposure to rats. Most of the radioactivity was excreted via the urine (66-76%), feces (13-21%), and CO_2 (8%). Very little (2%) was exhaled as DCP. At the end of 72 hrs, only 2-3% of the dose remained in the carcass. More than 90% of an oral dose was absorbed. Rats can now be exposed to varying concentrations of DCP by inhalation.

2) Studies on the disposition of methyl bromide (MB), a component with varying industrial uses including manufacturing of pharmaceuticals and dyes, as a refrigerant, and as a fumigant of soil, commodities, and stored grains, have begun. Appropriate methods for vapor generation and quantitation were developed. Recovery of an oral and ip dose has been analyzed, with little radioactivity persisting in the body by 3 days after exposure. Effects of varying doses on the disposition of inhaled MB can now be investigated.

3) Azodicarbonamide (ADA) has been nominated for inhalation testing by the NTP because of its widespread potential for occupational exposure. A number of current instances of workers developing asthmatic conditions upon exposure to ADA have been reported. Before any inhalation studies can begin, it is imperative to determine if an appropriate aerosol can be generated and at what concentration. Using a fluidized bed generator after milling, an aerosol with a concentration of approximately 160mg ADA/m^3 and a mass median aerodynamic diameter of 3.9 μm could be produced. This should result in an approximate pulmonary deposition of 1.2 mg ADA/rat using a 6 hr exposure. Studies are being conducted to determine if higher concentrations of ADA aerosol can be generated without increasing the particle size.

PROPOSED COURSE:

1. A study of the deposition, disposition and metabolism of inhaled azodicarbonamide will be done in the rat.

2. Additional compounds will be studied as requested by personnel in the NTP or in the NIEHS Intramural Program.

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 if 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.

RESEARCH TRIANGLE INSTITUTE - Research Triangle Park, NC 27709
(NO1-ES-1-5007)

TITLE: "Pharmacokinetics of Xenobiotics"

CONTRACTOR'S PROJECT DIRECTOR: A. Robert Jeffcoat, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: July 15, 1981

CURRENT ANNUAL LEVEL: $250,388

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 fate of 1,3-dichloro-5,5-dimethylhydantoin (Cl_2DMH) was studied in the rat. Cl_2DMH rapidly gives up chlorine in solution and equilibrates to give a mixture of 1,3-dichloro- and 1-chloro-5,5-dimethylhydantoin. Thus it was established that animal or human exposure to Cl_2 DMH would necessarily be an actual exposure to a mixture of the mono- and dichloro-products. The metabolism and disposition of that mixture is being studied in the rat.

2) Toluene 2,6-disocyanate (2,6-TDI) has been studied in vivo and in vitro. At low concentrations 2,6-TDI hydrolyzes sequentially to 2-amino-6-isocyanate-toluene then to 2,6-diaminotoluene. At higher concentrations it polymerizes. In vitro experiments established that the half-life of 2,6-TDI in serum was less than 30 sec and less than 2 min in stomach contents. In vivo experiments indicated that high oral doses, 900 mg/kg, of 2,6-TDI polymerized in the stomach. A larger portion of a low dose, 60 mg/kg, was absorbed but most of the dose polymerized in the stomach, was not absorbed and was excreted in the feces. That portion of the dose which was absorbed was hydrolyzed to 2,6-diaminotoluene and excreted in urine.

3) In vitro studies established that ethyl acrylate (EtOAcry) was metabolized in vitro by blood (half-life ca. 13 min), and less rapidly by forestomach tissue, glandular stomach tissue and stomach contents from both male and female Fischer 344 rats. The metabolism fitted first order kinetics with respect to EtOAcry in all cases although the metabolism of EtOAcry by blood appears to be more complex. In vivo, concentrations of non-protein thiols in the forestomach and glandular stomach were substantially reduced 30 and 120 min after a single oral dose of 100 mg/kg or 200 mg/kg of EtOAcry in corn oil. Thirty minutes after dosing, less than half of the dose remained in the stomach as parent compound. No differences were observed between males and females with respect to in vitro metabolism, decreased non-protein thiols or the rate of disappearance of EtOAcry from stomach. Ethyl acrylate was found in blood from the portal vein at concentrations up to 27 µg/mL in all animals dosed with 200 mg/kg of EtOAcry, but not in intraoccular blood (limit of detection was 1 µg/mL). Therefore, it is assumed that EtOAcry is absorbed from the stomach, but rapidly degraded in blood and possibly liver so that it does not enter the perpherial circulation.

4) The stability and fate of the organic peroxide t-butyl perbenzoate (TBP) was established in vitro and in vivo. The compound is stable in the dosing solution, corn oil, and in the presence of protein, bovine serum albumin. However, TBP is rapidly hydrolyzed by enzymes in plasma and liver. Other, tests currently underway will determine the stability of this compound in the gastrointestinal tract and on skin and determine its fate following absorption. The major metabolites/breakdown products of TBP will also be isolated and identified.

PROPOSED COURSE: 1) Work on t-butyl perbenzoate and 1,3-dichloro-5,5-dimethylhydanoin will be completed.

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 or 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 - Birmingham, Alabama 30332
(NO1-ES-1-5008)

TITLE: "Pharmacokinetics of Xenobiotics"

CONTRACTOR'S PROJECT DIRECTOR: Donald L. Hill, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: July 15, 1981

CURRENT ANNUAL LEVEL: $239,424

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-livers, 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, sacrified 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 realtive 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 gastrointestinal absorption of a series of pigments was studied in rats. Each of these compounds, C.I. Pigments Red-3, Red-23 and Yellow-74 was poorly absorbed and excreted exclusively in feces. Supplemental studies to determine if pigments could be metabolized or otherwise degraded in vitro by liver, feces or intestinal contents indicated that they were stable under biological conditions.

2) The absorption and distribution of amsonic acid was studied in the male Fischer 344 rat at a high-dose of 1540 mg/kg and a low-dose of 145 mg/kg. No detectable amsonic acid was detected in plasma, whole blood, or liver. At 1 hr and 4 hr after dosing, most of the compound was present in the gut contents; and, at 24 hr, most was in the feces. A small amount found in urine was probably due to contamination of the urine by feces. Only those tissues directly in contact with the compound contained detectable amounts. Since there was no significant difference in the percent recoveries at 1, 4, and 24 hr after dosing (or, in a separate experiment, at 0 time), it is unlikely that appreciable metabolism or absorption occurred.

3) 2-Mercaptobenzimidazole, which is used in the rubber processing industry, is a structural analog of the known carcinogen, ethylenethiourea. Fischer 344 rats were administered a high oral dose (50 mg/kg), a low oral dose (0.5 mg/kg), or an intravenous dose (0.5 mg/kg) and placed individually in glass metabolism cages. Urine, feces, and exhaled CO_2, were collected at 24, 48, and 72 hr and tissues at 72 hr. Samples of each collection were assayed for radioactivity. There was little dose related difference in the disposition of radioactivity. This compound was readily absorbed and rapidly metabolized. Liver and kidney contained the highest concentrations of radioactivity. A major metabolite had the same retention time as benzimidazole on HPLC.

4) Studies are underway to evaluate the differential disposition of [^{14}C]9-aminoacridine, administered dermally or intravenously.

PROPOSED COURSE: 1) Studies of the dermal absorption and disposition of 9-aminoacridine 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 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

El Dareer, S.M., Noker, P.E., Tillery, K.F., and Hill, D.L.: Investigations on the basis for the differential toxicity of hexachlorocyclopentadiene administered to rats by various routes. J. Toxicol. Environ. Health, in press.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC. - Columbus, Ohio
(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: $70,000

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 or reproductive toxicity.

METHODS EMPLOYED: Approximately twenty 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 COURSE: 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 were sent to EHRT over the remainder of the contract; the protocol was modified as the testing continues. Data were collected and sent to the chemical managers.

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. - Columbus, Ohio
(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: $253,705

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 been completed for eight chemicals, reports are being prepared and will start in 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 - Research Triangle Park, North Carolina
(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: $272,013

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to evaluate a new reproductive toxicology testing system.

METHODS EMPLOYED: This reproductive toxicology testing system employes 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 been completed for eight chemicals and reports are being prepared and will start in 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.

ENVIRONMENTAL HEALTH RESEARCH AND TESTING, INC. - Columbus, Ohio
(NIH-NO1-ES-2-5026)

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: May 1, 1983

CURRENT ANNUAL LEVEL: $82,933

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 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 COURSE: 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.

NORTHROP SERVICES, INC. - Research Triangle Park, North Carolina
(NIEHS-NO1-ES-79-0009)

TITLE: Animal Research on the Inhalation Toxicology of Environmental Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Bernard Adkins, Jr., Ph.D.

PROJECT OFFICER (NIEHS): E.W. Van Stee, D.V.M., Ph.D.
Head, Inhalation Toxicology Group, STB, TRTP

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL (5 years): $2,778,653.00

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 because 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 and place into operation a computer-assisted augmentation of the existing gas inhalation facility based on concepts and specifications provided by the Government. Conduct a 2-year oncogenesis study in which groups of rats and hamsters receive NO2 by inhalation and 2,6-dimethylmorpholine (DMM) in the drinking water, air plus DMM, NO2 and plain drinking water, or air plus plain drinking water. Install a chamber effluent scrubbing system based on the use of activated charcoal cannisters. Conduct a 2-year chronic toxicity test on napthalene and wollastonite. Conduct 6-month inhalation exposures of rats and mice to ethylene dibromide, ethylene oxide, ethylene dichloride, vinyl chloride, and vinylidene chloride.

MAJOR FINDINGS AND PROPOSED COURSE: Site preparation by the Government is complete and the development of the computer system is continuing. Computer-assisted operation of the facility should be well along by 1984. A detailed protocol for the 6-month exposures to the group of 5 chemicals has been written and reviewed. The NO2-DMM exposures have been completed but the final data are not yet available for use in preparing the final report. Exposures of Strain A mice to ethylene oxide and vinyl chloride have resulted in concentration-dependent increases in the rate of formation of pulmonary adenomas.

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 into closer alignment with 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 NO2 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 NO2 by inhalation has been reported by NIEHS. The results of these studies demonstrate the existence of a specific, chemical link between exposure to NO2 and the in vivo formation of potentially carcinogenic nitrosamines.

UNIVERSITY OF ARIZONA - Tucson, Arizona 85724
(NO1-ES-8-2130)

TITLE: "Pharmacokinetics of Xenobiotics"

CONTRACTOR'S 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 dermal absorption of a series of phthalate esters and phthalic acid was studied in Fischer-344 rats. Three dose levels 5 mg/kg, 50 mg/kg, 166.7 mg/kg, respectively) of diethylphthalate did not show any significant differences in their excretion profile or the amount remaining on the skin. The absorption low molecular weight compounds was quite rapid with 23-34% of the dose appearing in the urine in the first 48 hr (as compared to 30-49% total excretion in the urine in 7 days). In contrast, experiments with di (2-ethylhexyl)phthalate showed only 5% had appeared in urine and feces in the first 5 days and the remainder remained on the skin.

2) Diallylphthalate (DAP) caused liver and kidney toxicity in Fischer 344 rats, but not in B6C3F1 mice during a 13 week subchronic study (Litton Bionetics project no. 10608). To determine if the disposition and metabolism of DAP relate to the differential toxicity, Fischer 344 rats and B6C3F1 mice (n=3) were given oral doses of ^{14}C DAP, 1, 10 or 100 mg/kg or 10 mg/kg i.v. and placed in metabolism cages for 24 hr. The exhalation of $^{14}CO_2$ and volatile metabolites was determined overtime, and the cumulative urinary and fecal excretion was measured. In rats, 25-30% of the DAP was excreted as CO_2 while 50-70% appeared in the urine and 2-5% in the feces at the end of 24 hr. In mice, 6-12% of the

DAP was excreted as CO_2, and 80-92% was excreted in the urine. Metabolites present in the urine of both species were monoallylphthalate (30% of the dose) and allyl alcohol. Solvent extraction and TLC revealed the presence of polar metabolites that were different between the rat and mouse. In addition, the non-extractable metabolite fraction differed (8 and 20% in rats and mice, respectively). This qualitative and quantitative difference in metabolism of DAP may explain the species difference in toxicity.

3) The influence of pharmacokinetic parameters on toxicity of the neurotoxic tri-o-cresyl phosphate was studied by comparing it with its isomers, tri-m-cresyl (TMCP) and tri-p-cresyl phosphate (TPCP). Rats (n=3) were adminstered ^{14}C labeled compound by gavage at 2,20, 200 mg/kg. For all isomers and dosages, 90-100% of the administered ^{14}C was recovered in the urine and feces within 3 days. Rats receiving TOCP at 2 and 20 mg/kg eliminated 90% of the dose (60-70% urine, 20-25% feces), however at 200 mg/kg only 60% of the dose had been eliminated (45% urine, 16% feces) by 24 hr. For TMCP, equal amounts of the 20 and 200 mg/kg dose administered appeared in urine and feces (24% and 12%, respectively) by 24 hr. By 3 days the major route of excretion for the 20 and 200 mg/kg dose was via the feces while there was equal elimination by both routes for 2 mg/kg. The excretion route and rate of TPCP also showed dose dependence, with 59, 36 and 17% of the dose (2, 20, 200 mg/kg, respectively) appearing in the urine and 25, 46 and 47% appearing in the feces by 24 hr.

4) Absorption and excretion studies with gallium arsenide have been completed in rats by oral and intratracheal routes of exposure and show that about one-tenth as much arsenic was absorbed after oral administration as compared to intratracheal dosing. Between 70-80% of the arsenic in the lungs after intratracheal dosing was either absorbed or cleared from the lungs. Indices of GaAs toxicity observed at levels as low as 100 mg/kg were decreased body weight, increased lung wet weight and inflammatory response in lung as measured by histopathology.

5) To further investigate the effect of structure on metabolic rate and disposition research has focused on the metabolism and distribution of three PCB congeners, 4,4'dichlorobiphenyl (4-DCB), 2,2',3,3',6,6'-hexachlorobiphenyl (236-HCB), and 2,2',4,4',5,5'-hexachlorobiphenyl (245-HCB) in rat hepatocyte suspensions. Results of this work demonstrate that the limited metabolism of 4-DCB and 236-HCB observed in hepatocyte suspensions was not due to a destruction of the mixed function oxidases or to a depletion of cofactors byt possibly represented nonspecific binding or partitioning of the PCB into subcellular compartments thereby affecting the amount of substrate available for metabolism. Kinetic studies were performed to examine the rates of metabolite production of 4-DCB (0.1-300 μM) and 236-HCB (0.1-300 μM). Each congener was metabolized by two Michaelis-Menten processes. Both a high affinity (4-DCB: K_m 1.05×10^{-6}M, M_{max} 8.43×10^{-13}M; 236-HCB: K_M 4.78×10^{-7}M B_{max} 3.77×10^{-12}M) and a low affinity site (4-DCB: K_m 3.4×10^{-4}M, B_{max} 5.68×10^{-11}M; 237-HCB: K_m 6.45×10^{-4}Nm/b_{max} 3.67×10^{-10}M) exist.

These results indicate that the metabolic potential of a PCB congener is influenced by both the affinity of the substrate for cytochrome P-450 and the subcellular distribution of substrate and not by the formation of a metabolite stable complex. The high proportion of radiolabel remaining in the cytosolic

fraction following equilibrium dialysis and subcellular distribution studies indicates the presence of binding protein(s).

6) To determine how man handles polychlorobiphenyls we are comparing the in vitro metabolism of 2,2',3,3',6,6'- and 2,2',4,4',5,5'-hexachlorobiphenyl (236-HCB and 245-HCB) and 4,4'-dichlorobiphenyl (4-DCB) by human, monkey and dog liver microsomes.

The qualitative metabolism of 245-HCB, 236-HCB and 4-DCB by monkey and human liver microsomes is very similar and is consistent with previous in vivo work. The metabolites of the microsomal metabolism suggest the formation of an electrophilic intermediate which we have shown to be capable of covalent binding to microsomal protein. Quantitatively, monkey liver microsomes metabolize 236-HCB and 4-DCB faster than human liver microsomes. Monkeys have a four-fold higher concentration of cytochrome P-450 per milligram microsomal protein than humans, suggesting that monkeys will excrete 236-HCB and 4-DCB faster than humans.

7) Disposition and metabolism of p-chlroaniline was studied in dogs and mice. The object of this study was to evaluate the importance of the N-acetylation of PCA to p-chloroacetanilide (PCAA) as a major pathway for the detoxification and elimination of PCA from the organism. Studies conducted in F-344 rats showed that after an i.v. dose of PCA, N-acetylation occurs rapidly, possibly as a first step in its metabolism and excretion. This study was conducted in two animal species which are poor N-acetylators of aromatic amines (dog and A/J mice) and one strain of mice (Swiss-Webster) which possesses both blood and liver N-acetylating activity. It appears that PCA is eliminated rapidly from all those species examined, regardless of their ability to N-acetylate aromatic amines. However, the persistence of radiolabel seen in the RBCs of the rat, which is not apparent in either the dog or mouse, may be related to the N-acetylation of PCA.

8) Kaplan and Murphy reported age-related differences in onset of acrylamide (ACMD)-induced toxic signs in multiple-dosed male Holtzman rats (Kaplan and Murphy, Toxicol. Appl. Pharmacol. 22, 259, 1972). We found on multiple dosing of 5- and 11-week old Holtzman rats, higher tissue concentrations of ACMD (as ^{14}C) were in 11-week olds vs. 5-week olds. It is hypothesized that these differences were an artifact of dose calculation (per kg body weight), exaggerated by the decreased tissue:body weight ratios calculated from 11-week olds vs. 5-week olds. This was supported by the lack of any age-related effect on 24 hr clearance of a single ip dose of ACMD.

PROPOSED COURSE: This contract will expire prior to FY-84.

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 dispostion and kinetic data to complement studies of toxic xenobiotics which will be done under the NTP or in the NIEHS Intramural Program.

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. 65:264-272, 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.

Miller, M.S., Miller, M.J., Burks, T.F., and Sipes, I.G.: Altered retrograde axonal transport of nerve growth factor after single and repeated doses of acrylamide in the rat. Tox. Appl. Pharm. 69 (1983) In press.

Schnellmann, R.G., Putnam, C., and Sipes, I.G.: Metabolism of 2,2',3,3',6,6'-hexachlorobipheny and 2,2',4,4',5,5'-hexachlorobiphenyl by human hepatic microsomes. Biochemical Pharmacol. In press.

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 the 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 and office automation activities.

COMPUTER TECHNOLOGY BRANCH

COMPUTER TECHNOLOGY BRANCH
Summary Statement

The Computer Technology Branch has the responsibility of providing computing, data processing, and office automation support to NIEHS and the National Toxicology Program. This service may be thought of as consisting of four cooperating and interdependent efforts, namely computer operations and support programming, information systems development, computer engineering, and office automation development and management.

The computer operations and programming effort assumes the responsibility for maintaining NIEHS' two VAX 11/780 computers and a network of terminals connected to the various computers at NIH/DCRT, Parklawn, and NCTR, assisting the NIEHS community in its use of available computer systems, providing programming consultation services as required, providing 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 for the development of large, automated systems for both the Institute and the NTP. Institute projects include efforts on behalf of the Office of Administrative Management, the Extramural Program, and the Office of Program Planning and Evaluation. For the NTP, ongoing projects include the Chemical Information and Tracking System, the Toxicology Data Management System (in cooperation with the National Center for Toxicological Research), and management of the Carcinogenesis Bioassay Data System.

Provision of computer engineering support to the laboratories of the Institute is also made available 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 personal computers, minicomputers and microcomputers, 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.

Office automation support to the Institute is a newly acquired responsibility which requires coordination with and complements the above detailed activities. Selection and acquisition of over 30 stand-alone word processors has been accomplished, and ongoing related duties include management of the machines and provision of training for their users. The next phase of this effort will be to study the Institute requirements for such functions as electronic mail and messaging and electronic calendaring and to recommend and implement the acquisition of appropriate hardware and software to accomplish this end.

DYNAMAC CORPORATION - ROCKVILLE, MD 20852
(NOI-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, 1983

CURRENT ANNUAL LEVEL: $998,676

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 NTP Animal Bioasay Program.

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 856 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. Validated chemical analysis methods have been published; enrollment of children has been completed; a substantial number of the cohort is now three to four years old; the chemical analyses are essentially complete, and data analysis has begun.

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 of mutagenic substances in body fluids), alterations in metabolism caused by exposure to toxic substances (such as the effects of PCBs 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 prospective study of 300 non-contracepting women: a recently developed assay for human chorionic gonadotropin is being used to monitor pregnancies. A study of the validity of measuring spontaneous abortion risk by interview has been completed. A study using mathematical modeling and simulation of the influences of maternal age and gravidity on risk for spontaneous abortion has been published.

A case-control study of risk factors for chronic renal failure has begun; cases are drawn from four medical centers. The feasibility of several related projects, including retrospective follow-up of childhood lead poisoning, is being investigated. Demographic studies, using Vital Statistics data and indirect exposure assessment, have been published on distribution and time trends for liver cancer mortality, occupation and lung cancer, and risk factors for breast cancer in Japanese and American women. Ongoing studies of this type include epidemiology of pleural plaques, risk factors for chronic renal disease mortality, and variation in cancer risk by national origin. Data collection for a case-control study of adult cancer risk in relation to smoking during pregnancy has been completed; initial analyses have focused on data quality (data were collected from study subjects and their parents) and overall cancer risk associated with passive smoking. Small projects on the etiology and histopathology of Reye Syndrome are in the field.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43001-11 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Demographic Investigations of Potential Human Health Hazards

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Dale P. Sandler Senior Staff Fellow 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; Department of Medicine, University of North Carolina Medical School

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.20	.45	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of vital statistics, demographic and other population data often leads to important clues linking environmental exposures to specific diseases. 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. Recently completed research includes the development of a statistical model for breast cancer risk which uses vital statistics and other data on breast cancer incidence and published data from several sources on the distributions of known breast cancer risk factors. In addition, the influence of environmental and occupational factors on mortality from chronic renal failure continues to be explored using mortality data for U.S. counties, census data, and data from the NIOSH occupational hazards survey. Data from the Health and Nutrition Examination Survey are being used to develop a new estimate of the number of persons who have been exposed to asbestos and to relate x-ray abnormalities to other measures of lung function. Existing data, including vital statistics, are also being used to make estimates of the clinical significance of findings from other epidemiologic studies.

Principal Investigator and All Other Personnel Engaged on the Project:

Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
David G. Hoel	Program Director	BRAP	NIEHS
Walter J. Rogan	Medical Officer	EB	NIEHS
Carl A. Keller	Epidemiologist	EB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Potential environmental hazards are studied using available demographic, vital statistics, and other population data. (1) Analysis of data related to breast cancer risk continues using incidence and mortality data for the U.S. and Japan and data on the distributions of known breast cancer risk factors. Other ongoing efforts include (2) analysis of occupational and demographic factors associated with mortality from chronic renal failure in U.S. counties; (3) analysis of the association between percent foreign born and cancer mortality in U.S. counties; (4) analysis of x-ray data from the Health and Nutrition Examination Survey to estimate the size of the U.S. asbestos worker cohort; (5) estimation of the clinical significance of findings from epidemiologic studies using vital statistics data and cost-benefit analyses.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Analysis of breast cancer data has led to the development of a statistical model for breast cancer risk which involves the concept of 'breast tissue age'. This model incorporates known breast cancer risk factors such as ages at menarche, first birth and menopause. The model, when applied to U.S. and Japanese data, appears to explain much of the difference in breast cancer rates between the U.S. and Japan. (2) Chronic renal failure mortality appears to be greater in the Southeastern United States. Ongoing analyses are designed to identify particular occupations that might be associated with increased risk. Preliminary analysis indicates that mortality is greatest in counties with lower educational levels, moderate average incomes and moderate population size. (3, 4) Analyses related to percent foreign born and cancer mortality and to estimation of the size of the asbestos cohort are ongoing. Data from the Health and Nutrition Examination Survey should prove particularly useful in that x-ray evidence of pleural plaques or pleural thickening should provide an accurate indication of asbestos exposure. (5) Individuals who have undergone gastric resection for ulcer disease are reported to be at two or three-fold risk for developing cancer in the gastric remnant. Screening recommendations have been based on this finding, but most studies were conducted in Scandinavian countries with much higher rates of gastric cancer. Analyses currently being conducted indicate that the low risk of gastric cancer in the United States and other pertinent factors make generalized screening less effective in the U.S. Revised screening recommendations are being developed. Similar analyses have been carried out to evaluate screening for second malignancies in women with irradiation for gynecologic tumors and to evaluate the role of carcinoembryonic antigen monitoring in colorectal cancer screening.

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.

PUBLICATIONS

Sandler, D.P., Sandler, R.S., and Horney, L.F.: Primary liver cancer mortality in the United States. J. Chronic Diseases 36: 227-236, 1983.

Milne, K.L., Sandler, D.P., and Everson, R.B.: Lung cancer and occupation in Alameda County: A death certificate case-control study. Am. J. Indust. Med. 4: 565-575, 1983.

Hoel, D.G., Wakabayashi, T., and Pike, M.C.: Secular changes in menarche, first birth and menopause in Hiroshima and Nagasaki, Japan. Am. J. Epidemiol. 82: 233-245, 1983.

Sandler, R.S., and Sandler, D.P.: Radiation induced cancers of the colon and rectum: Assessing the risk. Gastroenterology 84: 51-57, 1983.

Sandler, R.S., Freund, D.A., Herbst, C.A., and Sandler, D.P.: Cost-effectiveness of postoperative carcinoembryonic antigen (CEA) monitoring in colorectal cancer. Cancer, in press.

Pike, M.C., Krailo, M.D., Henderson, B.E., Casagrande, J.T., and Hoel, D.G.: 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. Nature 5920: 767-770, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43002-07 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Breast Milk and Formula Project

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Walter J. Rogan Medical Officer 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Widespread contamination of human milk by polychlorinated biphenyls (PCBs) and 1,1-(p-chlorobiphenyl)-2,2-dichloroethane (DDE, a metabolite of the pesticide DDT) is well documented, but illnesses resulting from such exposure to nurslings are essentially unstudied. This project involves: (1) development of sampling and PCB/DDE analysis methods for breast milk and other tissues and fluids that are reliable, reproducible, and contaminant free; (2) establishment and follow-up of a cohort of children for whom analyzed samples of milk and clinical data are available; (3) development of alternate methods of chemical analysis that are faster or cheaper than gas liquid chromatography; (4) evaluation of the children for specific outcomes thought to be related to DDE/PCB exposure; (5) data cleanup, editing and analysis.

Principal Investigator and All Other Personnel Engaged on the Project:

Walter J. Rogan	Medical Officer	EB	NIEHS
Beth C. Gladen	Statistician	SBB	NIEHS
James D. McKinney	Research Chemist	LEC	NIEHS
Richard B. Everson	Epidemiologist	EB	NIEHS
Thomas K. Wong	Staff Fellow	EB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is a prospective, birth cohort, follow-up study. Three North Carolina clinics contribute subjects; field personnel enroll subjects, administer questionnaires, collect samples, examine the children and their medical records, and administer standard behavioral and developmental tests. Visits to the study happen at term, and when the child is 6 weeks, 3, 6, 12, 18, and 24 months old, and then yearly. Colostrum/breast milk or formula, maternal and cord blood, and placenta are collected at term; maternal blood at 6 weeks post partum; breast milk at each visit until lactation ceases; formula to 6 months.

These samples are subjected to gas chromatographic and neutron activation analysis for PCBs, DDE, total organic 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.

In the study, 856 children are being followed; the oldest was born in April, 1978, the youngest in October, 1982. Validation of the chemical methods is complete, and results of those experiments are published or in press. Almost all samples have been analyzed. Cleanup, editing, and analysis of the data continues. Plans for followup of the cohort after 5 years of age are being finished; right now, we expect to convert to an address registry with mail or phone contact, supervised by staff of the epidemiology support services group.

MAJOR FINDINGS AND PROPOSED COURSE: We find 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. Twelve women in the study were exposed to a PCB spill; we were able to detect the kind of PCBs from the spill in their milk samples with higher than expected frequency, but the exposure was not sufficient to raise the levels overall.

The major remaining objectives are cohort preservation, data analysis, and reporting of results. We have specific hypotheses about morbidity, growth and development to test. Hypotheses about long term effects and the description and validation of alternate chemical methods are lower priority. Of the original study hypotheses, only behavior in school and school achievement lack

some data, but this should be obtainable simply from the registry. We have also begun to look at biochemical markers of exposure to environmental chemicals by analysis of placental mixed function oxidase enzymes. This work is in development stages now.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS 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 PCBs, in humans is important.

PUBLICATIONS

Rogan, W.J.: Epidemiology of fat soluble contaminants in the food chain. In Finberg L. (Ed): Chemical and Radiation Hazards to Children: Report of the Eighty-fourth Ross Conference on Pediatric Research. Columbus, Ohio: Ross Laboratories, 1982, 29-37.

Rogan, W.J.: PCBs and cola-colored babies: Japan 1968, Taiwan 1979. Teratology 26: 259-262, 1982.

Rogan, W.J., and Gladen, B.C.: Monitoring breast milk contamination to detect hazards from waste disposal. Environ Hlth Perspec 48: 87-92, 1983.

Rogan, W.J., Gladen, B.C., McKinney, J.D., Albro, P.W.: Chromatographic evidence of polychlorinated biphenyl exposure from a spill. JAMA 249: 1057-8, 1983.

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McKinney J.D., Abusamara A., Reed, J.H.: Detection of organically bound chlorine and bromine in human body tissues by neutron activation analysis. Analytical Chemistry 55: 91-6, 1983.

McKinney, J.D., Moore, L., Prokopetz, A., Walters, D. B.: Validated extraction and clean up procedures for polychlorinated biphenyls and 2,2(4 chlorophenyl)-1, 1 dichloroethene in human body fluids and infant formula. J Soc Off Anal Chem, in press.

Rogan, W.J., Gladen, B.C.: Potential morbidity from exposure to PCBs during pregnancy and via milk. Environ Hlth Perspec, in press.

Rogan, W.J.: Intrauterine and breast milk exposures. In Finberg, L. Chemical Pollution and Children. CRC, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43004-05 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Environmental Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Dale P. Sandler Senior Staff Fellow EB NIEHS

COOPERATING UNITS *(if any)*

Bowman Gray School of Medicine/Baptist Hospital; Duke University Medical Center; University of North Carolina Medical School; Charlotte Memorial Hospital; 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:
2.25	1.25	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There are a number of chronic diseases with poorly understood etiologies that contribute substantially to the morbidity of human populations and result in significant expenditures of public health dollars. Environmental agents may produce some of this disease, and identification of associations between exposures and certain diseases would presumably lead to the prevention of morbidity. Other than cancer, few chronic diseases have received much attention in studies of environmental hazards, yet other diseases might be the more common, although less obvious or dramatic, results of exposure to such hazards. The Epidemiology Branch is working towards a program in environmental epidemiology that will address the role of environmental factors in the etiology of some less well studied chronic diseases or sources of exposure. The program currently includes a number of studies of chronic disease or cancer epidemiology. Foremost among these is a case-control study of risk factors for chronic renal failure and related work involving development of a renal disease classification scheme for use in etiologic studies. The feasibility of other studies that relate to risk factors for chronic renal failure is being explored. Other studies in the environmental epidemiology program include a recently completed study of the relationship of parental smoking and subsequent cancer development in adult offspring, and investigation into the etiology of Reye's Syndrome.

Principal Investigator and All Other Personnel Engaged on the Project:

Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Richard B. Everson	Medical Officer	EB	NIEHS
Walter J. Rogan	Medical Officer	EB	NIEHS
Allen J. Wilcox	Medical Officer	EB	NIEHS
Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) We have recently begun a study of risk factors for chronic renal failure. The study uses a case-control epidemiologic design to identify risk factors and to determine the relative importance of different exposures in determining disease risk. Cases are patients discharged from one of four participating hospitals with a new diagnosis of chronic renal disease (creatinine > 1.5) during the period September 1, 1980 to August 31, 1982. Controls are healthy persons selected from the community by random telephone screening who are similar to cases in age, race, sex and county of residence. Both cases and controls are interviewed by telephone using a structured questionnaire designed to elicit information regarding environmental and occupational exposures of interest. Of particular interest are lead, cadmium, solvents, analgesic drugs, non steroidal anti-inflammatory drugs and antibiotics. (2) We intend to use the medical records of patients included in the case-control study to develop a renal disease classification system that can be used in etiologic studies. A first step will be to develop an algorithm for classifying patients that uses clinical and laboratory data (but not pathology) that will discriminate between disease entities. A second, more empirical approach will involve assigning weights to various clinical, laboratory and pathologic findings in order to replicate the clinical decision making process. (3) Other related projects include determining the feasibility of long term retrospective follow-up of a cohort of individuals treated in the past for childhood lead poisoning and identifying suitable data resources to evaluate the contribution made by such factors as hypertension in accounting for renal disease morbidity. (4) A case-control study of adult cancer risk and parental smoking during pregnancy and childhood has been conducted using cancer patients from UNC Memorial Hospital and both randomly selected healthy controls and controls identified as friends of cases. Data on parental smoking have been collected from over 500 cases, 500 controls and 700 of their mothers or siblings. Data from mothers and siblings were collected to determine the reliability of parental smoking histories provided by adult offspring. (5) Ongoing projects concerning Reye's Syndrome include measurement of aflatoxin B1 levels in livers of children dying from Reye's Syndrome and other causes in the Southeast, Michigan and Ohio and an autopsy review of histopathology to pursue the question of specificity of microvesicular fat in the liver in the presence of central nervous system disease.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Case identification for the renal study has begun at the four participating hospitals. Approximately 30% of persons discharged with a diagnosis indicating chronic renal dysfunction have been qualifying as eligible cases for study. We expect to identify 800

study cases through this process. The study questionnaire has been developed and preliminary testing has been completed. Interviews with cases and controls are expected to begin prior to the end of the fiscal year. (2) The renal biopsies of over 500 patients in North Carolina have been identified. Many of these are from patients who will be included in the case-control study. We will obtain comparable clinical data for those not included and use the combined data to begin work on a classification algorithm. (3) These projects are in the developmental stage. (4) Data collection and preliminary analysis in the parental smoking study have been completed. Of 801 eligible cancer cases, 518 were interviewed, the remainder having either died or refused to participate; 309 friend controls and 209 random controls were also interviewed as were 371 relatives of cases and 351 relatives of controls. Preliminary analysis suggests that subject's answers provide a good indication of exposure for most qualitative measures of parental smoking. Current analysis concerns the relationship of cancer risk and smoking by either parent or by the subject's spouse. Preliminary results suggest an association with paternal and spousal smoking related to overall cancer risk. Site-specific analyses will also be conducted. (5) Thus far, the livers of 15 patients have been obtained for the case-control study of Reye's Syndrome. Only one in seven tested thus far has shown aflatoxin, and it was not aflatoxin B1. Current plans involve review of data from Mississippi suggesting an association between Reye's Syndrome and aflatoxin B1. Review of histopathology in a series of cases diagnosed as Reye's Syndrome or other serious childhood illnesses with CNS involvement indicates that microvesicular fat in the liver might not be specific to Reye's Syndrome. A second review series has been assembled to confirm results of the first review.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE 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 widespread 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

Goyer, R.A. and Rogan, W.J.: When does a biologic change indicate disease? Environ. Health Perspect., in press.

Rogan, W.J.: Exposure epidemiology: Novel approaches. Journal of Environmental Science and Health, A17;457-461, 1982.

Everson, R.B.: Identification of population sensitive to the impact of the by-product of technology transfer. In Proceedings of the Symposium of the Biomedical Impact of Technology Transfer, Cairo, Egypt, February 1980, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43007-04 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection of human exposure to mutagenic substances by analysis of body fluids

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard B. Everson Medical Officer EB NIEHS

COOPERATING UNITS *(if any)*

National Institute of Occupational Safety and Health; Department of Medicine, University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Laboratory methods necessary for detecting mutagenic substances in human body fluids were 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. Compared with unexposed subjects, no evidence was found for increased levels of mutagenic substances in urine specimens from cases of or individuals at high risk for cancer of the bilharzial bladder, cases of cirrhosis or other disorders affecting hepatic function, or individuals occupationally exposed to agents used for cancer chemotherapy. In addition, no association was found between maternal smoking and the presence of mutagens in amniotic fluid. In the latter two studies, however, careful analysis of assay results suggested low levels of mutagenic activity in urine and amniotic fluid specimens from most subjects compared with solvent controls.

Principal Investigator and All Other Personnel Engaged on the Project:

Richard B. Everson Medical Officer EB NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Mutagenicity studies used modifications of the bacterial mutagenesis assay developed by Ames and coworkers. Adaptations were required to assess the effects on the bacteria of toxic and growth factors in the test samples and to make optimal use of the small samples of amniotic fluid available. Because widespread use of this assay in the toxicologic literature enhances interpretability of results, emphasis was placed on minimizing modifications required for epidemiologic studies. The effect of toxicity and growth factors was studied by developing an objective procedure for quantitating the bacterial lawn. This involved removal of cylinders of agar from plates, homogenization of the cylinders to suspend bacteria, serial dilution, plating, and scoring the bacteria. Sensitivity of the assay for small test samples was increased by reducing the diameter of plates used in the assays and proportionately decreasing the amounts of agar and other reagents employed while keeping the quantity of the test sample constant.

MAJOR FINDINGS AND PROPOSED COURSE: Four studies have been completed. We have previously reported 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 the procedure for quantitating bacteria in the plate lawn described above to prevent falsely positive interpretation of assay results. A second previously completed study did not confirm a recent report indicating that mutagenic substances are present in urine samples from non-smokers with cirrhosis. Our results suggested the earlier findings may have been due to technical interference with the testing procedure by non-mutagenic materials in urine. New studies using these techniques found no evidence of increased excretion of mutagenic substances in urine specimens of pharmacists and nurses who prepared or administered mutagenic drugs used in the treatment of cancer. In addition, another study found no evidence of mutagens in human amniotic fluid specimens associated with maternal smoking or use of medications. In both of these last two studies evidence of a relatively small (50 to 100 percent) increase in the numbers of revertant colonies were found for most groups of subjects compared with assays of solvent controls. Although technical factors interfering with these assays cannot be totally excluded as the cause of increases of this magnitude, these results could not be attributed to increased numbers of bacteria in the background lawns or contamination during the collection or extraction procedures. Since technical interference seems unlikely, these results suggest the presence of low levels of mutagenic activity in these specimens. We are completing manuscripts describing this data and are investigating the feasibility of studies to determine whether these could be attributed to dietary sources.

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 51: 371-377, 1983.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43008-04 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and cellular environmental epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard B. Everson Medical Officer 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:
4.7	2.1	2.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the effective use of biochemical and cellular assays of human tissue and body fluid specimens in epidemiologic studies seeking evidence of adverse effects associated with specific environmental exposures. It emphasizes 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. Current effort focuses on the development of techniques for identifying genetic damage and alterations in metabolism associated with human exposure to potentially toxic substances. These techniques are being used in model studies of individuals exposed to known amounts of carcinogenic and mutagenic agents used for cancer chemotherapy, occupational groups, smokers, and individuals accidentally exposed to large quantities of PCBs. These studies are designed to help evaluate and refine assay and clinical methods, to investigate mechanisms involved in specific models of human disease, and to investigate the effects of exposures that may be important to public health.

Principal Investigator and All Other Personnel Engaged on the Project:

Richard B. Everson	Medical Officer	EB	NIEHS
Karsten Lundgren	Visiting Fellow	EB	NIEHS
Thomas K. Wong	Staff Fellow	EB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: Assays for detecting mutagenic substances in human body fluid samples have been refined and are being used to identify and evaluate exposures in field studies of occupational and other groups (see project Z01 ES 43007-04 EB). Several methods for detecting genetic damage itself are in different stages of investigation, including cytogenetic studies, assays for resistance to 6-thioguanine in human lymphocytes, and DNA strand breaks. Sensitivity, specificity, and other properties of individual assays as well as biologic relationships between these endpoints will be characterized by study of subjects with well characterized exposures, typically individuals with occupational or medical exposures. Later studies will emphasize use of the assays to identify genetic damage from less intense or less well defined environmental exposures. Effects of environmental exposures on metabolism are being studied by sensitive assays for certain of the major components of the mixed function oxidase (MFO) system in human placenta and mononuclear blood cells. Biochemical studies include fluorometric determination of benzo(a)pyrene hydroxylase and 7-ethoxycoumarin O-deethylase activity.

MAJOR FINDINGS AND PROPOSED COURSE: Findings for bacterial mutagenesis assays of human body fluids are discussed elsewhere (Z01 ES 43007-04 BB). Of the assays that could potentially be used to detect genetic damage, we are investigating the induction of DNA strand breaks using a recently developed assay that quantitates strand breaks by measuring loss of ethidium bromide-DNA fluorescence associated with unwinding of DNA in alkali. Assays have been done quantitating effects of radiation of human cells in vitro and effects of cancer chemotherapy in vitro and in vivo. Limitations in the replicability and sensitivity of this assay and difficulty in interpretating effects from DNA-crosslinking agents have prompted us to compare this method with other assays for DNA-strand breaks in future studies of similar model populations. Assay procedures for cytogenic studies have been standardized in our laboratory and a preliminary study seeking the likelihood of effects associated with the menstrual cycle on spontaneous levels of sister chromatid exchange and the extent of induction of SCE's by diethylstilbestrol have been initiated. Investigations of the relationships of cytogenetic alterations in somatic cells with preleukemia and other endpoints in populations treated with cancer chemotherapeutic drugs are being planned. In studies of effects on metabolism, over 100 placental samples have been assayed for benzo(a)pyrene hydroxylase and 7-ethoxycoumarin O-deethylase activity. As previously reported, 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. A study has been completed of an animal model designed to quantitate levels of gestational exposure to polychlorinated biphenyls necessary for induction of metabolic activity. In this model, activity in placenta, although

of less per unit tissue than in other organs, was induced at levels of exposure lower than doses causing induction in fetal liver but comparable to those required for induction of maternal liver. Manuscripts from these studies are near completion. Field studies using these assays have been designed both to determine whether environmental exposures (including smoking, diet, and accidental exposures to PCBs) are capable of affecting these markers for human metabolic function and the relative extent of environmental and genetic contributions to this response. Assay and clinical procedures have been decided upon and initial patient sampling is underway.

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, 1982.

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, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 44003-06 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Allen J. Wilcox Medical Officer EB NIEHS

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:
2.80	1.30	1.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reproductive epidemiology program emphasizes the development and application of new methods for measuring and analyzing human reproductive outcomes. Such outcomes include fertility, sub-clinical early fetal loss, spontaneous abortion, fetal growth, and birthweight. Each of these outcomes can be affected by environmental factors, and represents a possible endpoint for studying the effects of toxins on human reproduction. One major component of this project is a prospective study of early fetal loss among 300 women. A recently developed urine assay for human chorionic gonadotropin is being used to estimate the risk of early loss among these women. Daily urine specimens are being collected from women who have discontinued their use of birth control in order to become pregnant. This year the pilot study of 30 women has been completed and enrollment of the full sample is in progress. Risk of early loss will be studied in relation to common exposures in this population, such as use of alcohol, tobacco, caffeine beverages and medications. Another area of interest is the possible usefulness of measuring fertility through retrospective estimates of time to pregnancy. A feasibility study of this approach is under way. Work also continues on more theoretical problems in the analysis of spontaneous abortion risk and the analysis of birth weight.

Principal Investigator and All Other Personnel Engaged on the Project:

Allen J. Wilcox	Medical Officer	EB	NIEHS
Beth C. Gladen	Statistician	SBB	NIEHS
Clarice R. Weinberg	Statistician	SBB	NIEHS
Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Carl A. Keller	Epidemiologist	EB	NIEHS
Bruce C. Nisula	Medical Officer	DEB	NICHD

PROJECT DESCRIPTION

METHODS EMPLOYED: Reproductive outcomes may be sensitive endpoints 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 specific exposures. In particular, effort has focused on (1) measurement of human fertility as an endpoint in environmental studies; (2) a prospective study of subclinical early fetal loss and its relation to common exposures in a cohort of 300 women using a newly-developed assay for human chorionic gonadotropin; (3) refinement of methods for analyzing the risk of spontaneous abortion; (4) measurement of the validity of recall data regarding prior spontaneous abortion; and (5) development of a new analytic method for evaluating the effect of environmental hazards on birthweight.

MAJOR FINDINGS AND PROPOSED COURSE: (1) We have measured the accuracy of spontaneous abortion recall among 400 women, and found that nearly one-fifth of abortions occurring in the previous ten years are forgotten. These data provide a benchmark for evaluating the quality of recall data in other studies of spontaneous abortion. (2) We have begun a prospective study of very early pregnancy loss among 300 volunteers in an effort to measure the relation of such loss to environmental factors. The laboratory portion of this study includes the application of a newly-developed urine assay for human chorionic gonadotropin, being done in collaboration with NICHD. (3) A retrospective study of time-to-pregnancy as an estimate of fertility has begun, to test the feasibility of studying environmental effects using such data. (4) Further extensions of our work on the analysis of spontaneous abortion data and the analysis of birthweight data are in progress.

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., and Gladen, B.C.: Spontaneous abortion: the role of heterogeneous risk and selective fertility. Early Human Development 7: 165-178, 1982.

Wilcox, A.J.: Surveillance of pregnancy loss in human populations. Am. J. Industr. Med. 4: 285-291, 1983. (Also in Mattison, D.R. (Ed.), Reproductive Toxicology. New York, Alan R. Liss, 1983.)

Wilcox, A.J., and Russell, I.T.: Birthweight and perinatal mortality: I. On the frequency distribution of birthweight. Int. J. Epidemiol., in press.

Wilcox, A.J., and Russell, I.T.: Birthweight and perinatal mortality: II. On weight-specific mortality. Int. J. Epidemiol., 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.

Wilcox, A.J., and Russell, I.T.: Perinatal mortality: standardizing for birthweight is biased. Am. J. Epidemiol., in press.

Wilcox, A.J.: Intrauterine growth retardation: beyond birthweight criteria. Early Human Development, in press.

Keller, C.A., and Nugent, R.P. Seasonal patterns in perinatal mortality and prenatal delivery. Am. J. Epidemiol., in press.

STATISTICS AND BIOMATHEMATICS BRANCH

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

One of the most rapidly expanding efforts within the Branch has been applied statistical research support for the carcinogenesis studies conducted by 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. Use of logistic regression in the analysis of tumor incidence data is being examined as is the false-positive rate associated with two-year carcinogenicity experiments.

Risk assessment research is concerned with the modification and development of statistical procedures for using laboratory animal data to assess potential human risk associated with exposure to hazardous environmental agents. An extensive reanalysis of the Chemical Industry Institute of Technology laboratory study of the effects of formaldehyde exposure has been performed. This data base has also been used to evaluate some of the difficulties that often arise in the risk assessment process. Research is being conducted on the impact of pharmacokinetic consideration on low dose risk estimation. The determination of the most appropriate dosage scale (and the examination of other related issues) for species extrapolation in teratology is also being considered.

Statistical methodology development in the area of mutagenesis testing continues to be an important research activity of the Branch. Research attention is moving increasingly to large Ames Test data bases, such as the NTP data base with over 22,000 Salmonella experiments. In addition, cytogenetic studies of sister chromatid exchanges and chromosomal aberrations are being studied from the standpoint of modeling, comparing alternative statistical methodologies, and sample size determination.

In the area of population genetics current research 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 interactions of genetic recombination with DNA repair and normal meiosis at the molecular level. Much of this research is being carried out collaboratively with the Laboratory of Animal Genetics.

A variety of statistical research efforts in the area of Epidemiology have been conducted. These include the derivation of an index for synergy which does not depend on the rare disease assumption, the development of algorithms that improve the efficiency of screening programs, an examination of the effect of

confounding factors on the observed odds ratio from case-control studies, and the development of improved graphical displays for rate ratios.

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 determination, 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 computing work group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40004-06 SBB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods in Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Beth C. Gladen Statistician SBB NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

Principal Investigator and All Other Personnel Engaged on the Project:

Beth C. Gladen	Statistician	SBB	NIEHS
Takashi Yanagawa	Visiting Scientist	SBB	NIEHS
Walter Rogan	Medical Officer	EB	NIEHS
Clarice Weinberg	Mathematical Statistician	SBB	NIEHS

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) Suggestions were made for the improvement of graphical displays of rate ratios. (2) In analysis of data from a stratified follow-up study relating a dichotomous exposure to a dichotomous outcome, the usual procedure is to compute a weighted average of the stratum-specific relative risks. As an alternative, the pooled estimator based on collapsing across strata has been studied. An estimator of the variance of this estimate has been developed, and it has been demonstrated that the associated test yields better power than the usual method. Furthermore, frequency matching does not allocate controls optimally, and a rule for allocation has been developed based on minimizing the variance associated with the pooled estimator. (3) An index for synergy which does not depend on the rare disease assumption has been proposed. This index is essentially the nonadditive term in the log-nonresponse model sometimes used in toxicopharmacology. Based on enumeration of all possible tables for specified response probabilities, the test based on this index more closely approximates its nominal size than do tests based on other indices in use. (4) It is well known that a cross-sectional sample of people who have passed some transition point (e.g., onset of a chronic disease) will yield biased estimates of the age at the event. This is because a random sample of post-event people will be weighted towards those who experience the event early. An unbiased maximum likelihood estimation procedure has been developed for estimating the age-at-onset distribution, under the assumption that there are no birth cohort effects. (5) When screening a population, there are sometimes assays available that offer much greater sensitivity than needed. Algorithms based on pooling portions of specimens are being developed to improve the efficiency of such screening programs. Such an algorithm may be applied to pregnancy detection, using a new assay highly sensitive to HCG. (6) The estimation of incidence of a disease through the use of a diagnostic test was studied. The relationship between observed and true incidence was derived. The effect of varying sensitivity and specificity was studied. (7) The extent to which unmeasured confounding factors can disturb the observed odds ratio from a case-control study was assessed. Crude odds ratios were compared to the odds ratios that would have been observed in a matched study. The differences were found to be small.

The proposed course is to continue research efforts on statistical methodology problems that arise in connection with the epidemiological investigations carried out by the Institute.

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.J. and Gladen, B.C.: Spontaneous Abortion: The Role of Heterogeneous Risk and Selective Fertility. Early Human Development. 7:165-178, 1982.

Gladen, B.C. and Rogan, W.J.: On Graphing Rate Ratios. American Journal of Epidemiology. (in press)

Yanagawa, T., Kasagi, F. and Yoshimura, T.: A Method to Estimate Incidence Rates of Onchocerciasis with Consideration of False Negative Due to Skin Snip Biopsy. Biometrics. (in press)

Yanagawa, T. and Kasagi, F.: Estimating Prevalence and Incidence of Disease from a Diagnostic Test in: Recent Developments in Statistical Inference and Data Analysis Proceedings of the First Pacific Statistical Conference. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 40005-06 SBB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methodology and Analysis of Mutagenesis Testing Data

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Barry H. Margolin Mathematical Statistician SBB 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:
2.9	2.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long-term objective of this ongoing project remains the development of appropriate statistical techniques for the analysis of data arising from short-term assays under study by NTP. 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. Results are applicable to 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 tests. Work on in vivo and in vitro cytogenic assays continues.

Principal Investigator and All Other Personnel Engaged on the Project:

Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Ken Risko	Mathematical Statistician	SBB	NIEHS
Doug Simpson	Mathematical Statistician	SBB	NIEHS
Randy Tobias	Mathematical Statistician	SBB	NIEHS
Errol Zeiger	Head, EMTDP	CGTB	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Test for mutagenicity remains the dominant assay under study. Research attention is moving increasingly to large Ames Test databases such as: (1) the NTP database with over 22,000 Salmonella experiments conducted by three contract testing laboratories; and (2) the International Collaborative Study of Genetic Drift conducted by 38 laboratories testing 4NQO. A mixture of mechanistic and empirical modeling, together with statistical data analytic procedures, continues to be the main methodological approach toward these studies, as well as toward research on appropriate analyses of cytogenetic and transformation assays.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Based on the estimated mutagenic indices and measures of variability from the "genetic drift" study, it was concluded that if genetic drift contributed to the inter-laboratory variability in this international collaborative study, it was a minor component. As a rule, laboratories that reported "high" or "low" levels of spontaneous or induced revertants per plate, tended to deviate in the same direction for most strains and for both inhouse and reference cultures. (2) The statistical analysis of the 22,000 Ames experiments has recently been completed. Outliers were screened and the two main mechanistic models have been fitted to all experiments. Analyses of individual experiments have been combined, with suitable attention to multiple comparisons, so as to judge the mutagenicity of each compound. Preliminary indications are that a sequential strategy keying on the strain most likely to detect mutagens, namely TA100, will affect a significant savings in effort expended per compound tested without diminishing the detectability of Salmonella mutagens. (3) Data from recessive lethal tests with Drosophila are well described by Poisson sampling, save for a rare outlier. An outlier, typically referred to as a cluster, is attributable to a single premeiotic lesion that affects a number of the matings of the male in question. Classical outlier procedures have been implemented to exclude such clusters. The Poisson parameter has been shown to be homogeneous across experiments coincident in time within the same laboratory, but to be time inhomogeneous. Clear time trends were detected in a large control data set gathered over many years within one contract laboratory. (4) For the recessive lethal test, and comparative trials in general, the normal test has been shown to be preferred to the conditional binomial test tabulated by Kastenbaum-Bowman when the ratio of the sample sizes in the treated and control groups is between 0.80 and 1.25. (5) Recommendations have been offered for the value of the common sample size in the treated and control groups in a recessive lethal test that is needed to achieve a specified

power as a function of the specified false positive rate, the specified spontaneous recessive lethality rate and the assumed induced effect to be detected. These recommendations hold for comparative trials in general. (6) Preliminary indications are that in vitro SCE and aberration data are also Poisson distributed, and that there is no component of variability detectable from flask to flask under null conditions. The data from the transformation assays are currently awaiting similar analyses.

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

Margolin, B.H., Collings, B.J. and Mason, J.M.: Statistical analysis and sample size determinations for mutagenicity experiments with binomial responses. Environmental Mutagenesis, 5, 1983. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 41001-09 SBB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Risk Assessment Methodology Development

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

David G. Hoel Chief SBB NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Developmental Toxicology; Laboratory of Pharmacology; Developmental Biology Division, Health Effects Research Laboratory, EPA

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the development of statistical/mathematical methodology useful in the assessment of human health risks associated with exposures to potentially hazardous environmental agents. The primary focus is on the generation of improved statistical techniques for estimating adverse human health effects from laboratory animal data; and special emphasis is placed on dose-response modeling, low-dose extrapolation and species scale-up. Consideration is also given, on occasion, to the analysis and modeling of laboratory data for occupational/environmental agents of special interest. Present research efforts are particularly concerned with the impact of pharmacokinetic considerations on dose-response modeling in carcinogenesis and with the determination of the most appropriate dosage scale (and the examination of other related issues) for species extrapolation in teratology.

Principal Investigator and All Other Personnel Engaged on the Project:

David G. Hoel	Chief	SBB	NIEHS
Michael D. Hogan	Mathematical Statistician	SBB	NIEHS
Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Marshall W. Anderson	Mathematician	LP	NIEHS

PROJECT DESCRIPTION

METHODS EMPLOYED: In addressing various methodological issues that are involved in the assessment of human health risks, a variety of statistical and mathematical techniques are employed, ranging from empirical data evaluation and curve fitting to the generation of mathematical models that attempt to approximate biological mechanisms.

MAJOR FINDINGS AND PROPOSED COURSE: (1) An extensive reanalysis of the Chemical Industry Institute of Technology (CIIT) laboratory study of the oncological and toxicological effects of formaldehyde exposure was performed. This independent analysis raised some additional concerns about formaldehyde toxicity/carcinogenicity by demonstrating adverse effects at the lower experimental exposure levels and an increased risk of early death after censoring for nasal carcinomas. Although the causal mechanism for this latter effect is not known, the response is clearly dose related. (2) The CIIT data base was also used to evaluate some of the difficulties that often arise in the risk assessment process such as the problem of selecting an appropriate statistical procedure(s) to evaluate risk, of determining the proper exposure period upon which to base assessments, and of combining heterogeneous responses from different laboratory strains or species. (3) Research is continuing on the impact of pharmacokinetic considerations on low dose risk estimation. The main effort centers on the incorporation of more realistic features into pharmacokinetic models and the study of their effects on the estimation process. One generalization of pharmacokinetic models under consideration is the inclusion of competing nonlinear pathways. This kind of phenomenon is important since the disposition of a chemical and its metabolites can be very dose dependent. Another modification of these models that is being studied is the incorporation of differential cell turnover rates. (4) Preliminary evaluation of the species scale-up issue in teratology based on epidemiological and experimental data for the synthetic estrogen Diethylstilbestrol (DES) indicated that agreement between human and laboratory animal teratogenic responses may be maximized when interspecies comparisons reflect relative exposures during comparable developmental periods. Additional comparisons will be made for other known human teratogens for which sufficient data exists to permit similar evaluations. Nonhuman teratogens that are positive in two or more animal species will also be considered.

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

Hoel, D. G.: Statistical measures of risk. Drug Metab. Reviews 13: 829-838, 1982.

Hogan, M. D. and Hoel, D. G.: Extrapolation to man. In Hayes, A. W. (Ed.): Principles and Methods of Toxicology. New York, Raven Press, 1982, pp. 711-731.

Hogan, M. D.: Extrapolation of animal carcinogenicity data: Limitations and pitfalls. Environ. Health Perspect. 47: 333-337, 1983.

Brown, K. G. and Hoel, D. G.: Modeling time-to-tumor data: Analysis of the ED_{01} Study. Fund. Appl. Toxicol.: (in press).

Brown, K. G. and Hoel, D. G.: Multistage prediction of cancer in serially dosed animals with application to the ED_{01} study. Fund. Appl. Toxicol.: (in press).

Hoel, D. G.: The incorporation of pharmacokinetics in low-dose risk estimation. In Clayson, D., Krewski, D. and Munro, I. (Eds.): Toxicological Risk Assessment. Florida, CRC Press (in press).

Hoel, D. G., Kaplan, N. L. and Anderson, M. W.: Implication of nonlinear kinetics on risk estimation in carcinogenesis. Science 219: 1032-1037, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 44002-08 SBB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biomathematical Modeling

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Norman L. Kaplan 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:
1.6	1.6	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 interactions of genetic recombination with DNA repair and normal meiosis at the molecular level.

Principal Investigator and All Other Personnel Engaged on the Project:

Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Charles H. Langley	Research Chemist	LAG	NIEHS
Tom Darden	Staff Fellow	SBB	NIEHS

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) Work has continued on developing models relating to the evolution of transposable elements. Efforts have focused on analyzing a model which allows for elements to be defective and nondefective, i.e., the model is two-dimensional. Particular attention has focused on the time to extinction and the effects of population size. New work has been initiated directed towards the development of models which consider selective effects on the evolution of transposable elements. (2) Work has continued on the analysis of mitochondrial DNA sequence data within the context of nucleotide evolution. Recent data has suggested that certain regions of the genome are under strong selective constraints; and probably cannot easily undergo substitution. The large number of transitions as compared to the tranversions also appears to be a consequence of the substitutional process. Further work in this area will focus on the analysis of newly obtained data. (3) A mathematical model for the process of recombinational repair of DNA damage, and more generally for the interaction between DNA damage and recombinational mechanisms, has been developed. Based on the model, methods of analysis of alkaline sucrose sedimentation data have been developed in order to discern both levels and types of recombination associated with the presence of damage.

Methods are being developed to study the physical locations of sites of initiation of meiotic recombination.

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

Langley, C.H., Brookfield, J. and Kaplan, N: Transposable elements in Mendelian populations. 1. A Theory. Genetics, 1983.

Kaplan, N.L. and Brookfield, J.: Transposable elements in Mendelian populations III. Statistical Analysis. Genetics, 1983.

Kaplan, N. and Brookfield, J.: Effect on homozygosity of selective differences between sites of transposable elements. Theo. Pop. Biol., 1983.

Kaplan, N.: Statistical analysis of restriction enzyme map data and nucleotide sequence data. Statistical Analysis of DNA Sequence Data, p. 75-107. Marcel Dekker, 1983.

Kaplan, N. and Risko, K.: A method for estimating rates of nucleotide substitutions using DNA sequence data. Theo. Pop. Biol., 21: 318-328, 1981.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 45001-03 SBB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Design and Data Analysis Methodology for Animal Experiments

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Joseph K. Haseman — Research Mathematical Statistician — SBB — NIEHS
David G. Hoel — Chief — 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:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with statistical methodology issues involved in the design and analysis of animal experiments with particular emphasis on TRTP's two-year carcinogenesis studies. Research activities fall into three general categories: experimental design, statistical methodology development and data base analyses. Specific problems include: (1) modification of the experimental design employed in TRTP carcinogenicity studies, (2) use of logistic regression in the analysis of tumor incidence, (3) development of statistical procedures for incorporating historical control data in a formal testing framework, and (4) estimation of the false positive rate in two-year carcinogenicity experiments.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigators and All Other Personnel Engaged on the Project:

Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
David G. Hoel	Chief	SBB	NIEHS
Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Gregg E. Dinse	Staff Fellow	SBB	NIEHS
Takashi Yanagawa	Visiting Scientist	SBB	NIEHS

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 analytical test procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Various four dose designs (dependent on dose selections and animal allocations) of two year carcinogenicity screens were evaluated. After studying a variety of different dose-response curves based on linear, quadratic or linear-quadratic models, it was concluded that for a total sample size of 200 the following design was "optimal" (i.e., it maintained the power of the current design, while improving the design from the standpoint of goodness of fit and low-dose extrapolation): 50 controls, 30-40 animals at a dose of 20-30% of the maximum tolerated dose (MTD), 60-70 animals at a 1/2 MTD dose, and 50 animals at the MTD.

In evaluating tumor incidence data for incidental tumors, logistic regression has an advantage over the usual prevalence test in that it does not require time interval groupings. This eliminates the problem of deciding which set of intervals to use and allows an age-adjusted comparison of tumor rates in control and dosed groups even when differing mortality patterns result in little overlapping of death times. Preliminary simulation results suggest that in studies in which the test chemical adversely affects survival, the power of the logistic regression technique is greater than that of the customary prevalence test. The sizes of the different tests seem approximately the same.

Examination of historical control tumor rates indicates two major sources of variability: (1) calendar year (which is controlled to some extent by utilizing only the more recent historical control data) and (2) laboratory-to-laboratory variability. Certain tumors show evidence of variability among pathologists at the same laboratory. Animal supplier does not seem to be a major source of variability. A study of the relative merits of two procedures for incorporating historical control data in a formal testing framework has been completed. The results indicate that asymptotic procedures are unsatisfactory for rare tumors and that exact (conditional) tests should be employed.

Utilizing data from recent NTP feeding studies, the statistical significance of observed tumor increases was compared with the final interpretation regarding the carcinogenic effect of the chemicals under study. It was found that the scientific judgment process appeared to be closely approximated by a statistical

decision rule that regarded any P<0.01 high-dose effect (uncorrected for multiple comparisons) as a "biologically significant" increased tumor incidence (P<0.05 high-dose effect for rare tumors). It was shown that the actual overall false positive rate associated with this decision rule appears to be no greater than 7-8%.

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. Further study of logistic regression as an alternative to the incidental tumor test currently employed by the NTP will also be conducted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The TRTP's carcinogenesis testing 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

Dinse, G.E., and Lagakos, S.W. Regression Analysis of Tumor Prevalence Data. Journal of the Royal Statistical Society, Series C. (in press)

Haseman, J.K. Patterns of Tumor Incidence in Two-Year Cancer Bioassay Feeding Studies in Fischer 344 Rats, Fundamental and Applied Toxicology, 3: 1-9, 1983.

Haseman, J.K. A Re-examination of False-Positive Rates for Carcinogenicity Bioassays. Fundamental and Applied Toxicology. (in press)

Haseman, J.K., Huff, J.E. and Moore, J.A. Response to "The Lifetime Feeding Study in Mice and Rats -- An Examination of its Validity as a Bioassay for Human Carcinogens." Fundamental and Applied Toxicology. (in press)

Hoel, D.G. Conditional Two-sample Tests with Historical Controls. Contributions to Statistics: Essays in Honor of Norman L. Johnson, North Holland Publishing Company. (in press)

Portier, C. and Hoel, D.G. Optimal Design of the Chronic Animal Bioassay. Journal of Toxicology and Environmental Health. (in press)

LAWRENCE BERKELEY LABORATORY/UNIVERSITY OF CALIFORNIA
Berkeley, California 94620
(222Y01-ES-10066)

TITLE: Quantitative Species Extrapolation in Carcinogenesis

CONTRACTOR'S PROJECT DIRECTOR: Bruce N. Ames, Ph.D.

PROJECT OFFICER (NIEHS): David G. Hoel, Ph.D., Chief
Biometry and Risk Assessment Program

COLLABORATING INSTITUTE: Department of Energy

DATE CONTRACT INITIATED: April 1, 1981

CURRENT ANNUAL LEVEL: $155,000

PROJECT DESCRIPTION

OBJECTIVES: The quantitative assessment of factors that may lead to differential species responses to a carcinogenic exposure and the development of procedures for extrapolating carcinogenic risk projections across species have been hampered by the very limited availability of epidemiologic data suitable for quantitative interspecies comparisons. Therefore, this project was initiated in part to develop a large-scale laboratory animal data base for making non-human interspecies comparisons to evaluate quantitatively the impact that various factors have on the variability in species responses observed in long term carcinogenic screening studies.

METHODS EMPLOYED: (1) A set of acceptability criteria covering minimum sample size, use of concurrent controls, duration of experiment, length of exposure, route of administration, etc. were developed for evaluating studies reported in the literature and deciding which should be abstracted into a computerized data base. (2) Information from this data base is in turn being used to address a number of specific issues that bear directly on the quantitative extrapolation of cancer risk estimates across species. All intra and interspecies comparisons are based on the TD_{50}, i.e., the estimated dose required to give single risk tumor incidence of 50% at the end of a standard lifespan, as the index of potency. (3) The final phase of the project will involve a detailed comparison of potency indices for chemicals common to both the cancer bioassay and a related data base on Salmonella test results to determine if Salmonella test data can be used to develop realistic, quantitative estimates of carcinogenic potency.

MAJOR FINDINGS AND PROPOSED COURSE: (1) By the end of the first year of the Interagency Agreement an operational computer data base was constructed that included all "acceptable" NCI/NTP bioassays for which technical reports were published prior to July 1981 as well as the set of Weisberger experiments on aromatic amines. Further data from bioassays on these same compounds that were

also incorporated into the system. In addition statistical computer programs reported in the literature and that satisfied the acceptability criteria were were developed for calculating relative potency indices using information from the data file. (2) Preliminary analyses of the comparability of results for males and females and for mice and rats have been performed. The issue of species-specific target site has also been addressed. Variability in response associated with strain differences and route of exposure are currently under investigation. (3) Both the cancer bioassay and the Salmonella data base are still being analyzed, and the inter-test system potency index comparison will begin as soon as these analyses are completed.

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): Joseph K. Haseman, Ph.D.
Research Mathematical Statistician
Statistics and Biomathematics Branch, BRAP

DATA CONTRACT INITIATED: July 31, 1981

CURRENT ANNUAL LEVEL: $192,590

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 carcinogenesis experiments, pre-chronic studies, and certain other "special studies" with laboratory animals.

METHODS EMPLOYED: During this year of this contract, pre-chronic data from 20-25 studies were analyzed. These data were abstracted from reports prepared by a number of NTP contract laboratories (each summarizing results for a particular chemical under test). The variables of interest included organ and body weights, hematology and clinical chemistry parameters, and histopathology findings. For each chemical the data were computerized, pairwise comparisons and trend tests carried out, and summary statistical reports prepared. This information was then used for the selection of doses for the chronic study. In addition to these analyses, two special projects were initiated: (1) analyses of data from a series of special reproduction and fertility studies carried out by the NTP, and (2) summarization of sentinel animal data from NTP chronic and subchronic studies. The latter project is useful for detecting the presence or absence of viruses in the animal colonies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE NTP: This contract provides the statistical support necessary to effectively analyze large volumes of pre-chronic data generated from various NTP laboratory animal studies.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

During Fiscal Year 1983, the National Advisory Environmental Health Sciences Council reviewed 364 applications for which NIEHS was primary or secondary assignee. This represents a decrease of 263 applications over Fiscal Year 1982. One hundred and thirty-seven new and competing awards were made: 101 regular research grants, one Environmental Health Sciences (EHS) Center, three Research Career Development Awards, one Mid-Career Development Award, 15 Individual NRSAs and 14 institutional NRSAs. These new and competing awards, plus the non-competing awards, brought the 1983 total to 428 active grants, an increase of seven awards above Fiscal Year 1982.

The Grants Management Branch staff has been trained to use IMPAC and WYLBUR in preparation of grant award documents. The new automated data system now in use has reduced by several days the time necessary to issue grant awards. GMB staff is continuing to develop new lists and reports that will retrieve information from the IMPAC system in order to improve management efforts.

A new research development award, the "Clinical Investigator Award", in environmental health research was announced in FY '83. This five year award is designed to provide intensive guided research experience for clinicians. It will provide up to three years of guided research experience under an established researcher plus two years of additional support during which time the awardee will be expected to develop a program in Clinical research within his/her home department.

The first awards under this program are expected to be made in FY '84.

The Scientific Review Unit has assumed full responsibility for review of Center, program project, training, special development award and cooperative agreement applications. Contract proposals are reviewed according to the NIEHS guidelines for review of Contract proposals.

Fifty-eight research grant applications and nine contracts were reviewed in FY '83.

The Program Analysis' Computerized Information System (INTERIM) was completed and became operational. Departure of the resource person for maintenance of the system has delayed further refinement of this system. One of the staff in this unit is being trained with the cooperation of BRAP in the operation and maintenance of INTERIM.

HIGHLIGHTS OF RESEARCH IN EHS CENTERS

Harvard University

During the past several years the physiology department expanded until it constituted well over half of this center. This past year has seen an organizational restructuring within the Center in which the Radiobiology unit has joined with members of the current Microbiology Department to form a new Department of Cancer Biology, Biochemical Toxicology has become a free standing unit, and the remaining programs in the Physiology Department have joined with Environmental Health Sciences Department to form a new Department of Environmental Health, including both biologists and engineers. The Center serves as an integrating umbrella over these administratively independent units designed to foster formal and informal collaborative arrangements that cross departmental boundaries to serve the purposes of academic programs and multidisciplinary research projects.

Laboratory Studies:

Three groups of scientists use laboratory approaches to research and graduate training in environmental health sciences. These identify themselves as:

Radiobiology and Experimental Carcinogenesis

Biochemical and Environmental Toxicology

Respiratory Biology and Inhalation Toxicology

Radiobiology and Experimental Carcinogenesis:

The primary goal of this unit is better understanding of the cellular and molecular mechanisms of injury or impairment by environmental chemicals and by ionizing and non-ionizing radiation. The principal focus has been on cancer, both because it is an important end result of environmental exposures and because the mechanisms of carcinogenesis are poorly understood.

Specific aims of this program include the study of the induction of mutations, malignant transformation and cytogenetic changes in mammalian cells by physical and chemical carcinogens, and their correlations with specific DNA damage and repair processes. Recent studies have employed radioactive isotopes incorporated into DNA as probes for molecule lesions related to mutagenesis and transformation; the results suggest that DNA double strand breaks may be important in the induction of mutations by density ionizing radiation. Other studies have implicated free radical intermediates in the promotional phase of transformation in vitro. Another project is concerned with genetic susceptibility to cancer in human populations. Its overall aims are to identify and develop markers for hypersusceptibility to the mutational and carcinogenic effects of physical and chemical agents in the environment, based on the response of somatic cells to the effects of DNA damaging agents.

The phorbol ester tumor promoting agent 12-O-tetradecanoyl-phorbol-13-acetate (TPA) markedly enhances radiation induced transformation in vitro. The promoting effects of this agent occurred largely during the early, proliferative phase of expression, though irradiated cells retained the ability to respond to TPA promotion at long times after X-ray exposure. Other agents such as

Epidermal Growth Factor and interferon also promoted X-ray transformation in this system. The glucocorticoids cortisone and dexamethasone, as well as the non-steroidal anti-inflammatory agent indomethacin, induced low to moderate levels of transformation by themselves.

TPA alone has been shown to induce sister chromatid exchanges in mouse 10T½ cells and gross chromosomal aberrations in human lymphocytes. In both cases, the clastogenic effect of TPA appears to involve free radical intermediates, particularly superoxide anions, as it can be suppressed by concomitant incubation with superoxide dismutase.

Fibroblasts from a number of cancer prone disorders have been used to examine the kinetics of the change in the frequency of sister chromatid exchanges (SCE) during liquid holding recovery induced in noncycling cells by ultraviolet light. In normal fibroblasts, the frequency of SCE declines rapidly with recovery intervals of 12-24 hours reaching nearly spontaneous levels. Distinct abnormalities in this response have now been identified in fibroblasts from several cancer prone disorders. No decline in SCE with liquid holding recovery times of up to 96 hours has been consistently observed in cells from Gardner's syndrome patients in three different families. A significantly reduced rate of decline has been characterized by enhanced susceptibility to spontaneous and radiation-induced skin cancer. This is being extended to other cancer prone families in which both affected and non-affected individuals can be studied in order to determine whether the SCE effect is associated specifically with the gene carriers. This response is of particular interest, as it may more directly reflect the activity of a DNA repair process.

Biochemical and Environmental Toxicology:

This program is under the direction of Dr. Armen H. Tashjian, Jr. and is a fairly new program which started in 1979. Facilities have recently been expanded and upgraded, and the program now occupies two entire floors in the Health Sciences Research Building. Additional faculty are currently being recruited. The orientation of this program is mechanism-based and its goals are research and graduate training aimed at better understanding of the molecular mechanisms of toxicity of specific classes of important environmental chemicals.

It was shown that aflatoxin B_1 induces the G:C to T:A transversion in the lacI gene of Escherichia coli. This finding, coupled with earlier demonstration that three other carcinogens--benzo(a)pyrene, cyclopenta(cd)pyrene, and N-acetoxy-2-fluorenylacetamide--also specifically induce this transversion, have led to the hypothesis that the mutagenicity of these carcinogens derives from their ability to induce apurinic sites in DNA. Current work is directed, in part, at establishing the importance of apurinic sites in bacterial mutagenesis.

Serially cultivated keratinocytes of human and rat epidermis and esophagus were compared with respect to their sensitivity to toxic effects of 3-methylcholanthrene and ability to metabolize benzo(a)pyrene. 3-Methylcholanthrene was highly toxic to the human keratinocytes and to early passage rat epidermal keratinocytes as evidenced by markedly reduced growth upon continuous exposure or reduced colony forming ability after one day exposure to concentrations of 0.4 - 40 μM in the culture medium. Rat esophageal and late passage rat epidermal cells appeared insensitive to 3-methylcholanthrene by these criteria. All the cell types except late passage rat epidermal cells metabolized benzo(a)pyrene at comparable rates. Biotransformation in the human cells was greatly inhibited by β-naphthonflavone,

a specific inhibitor of aryl hydrocarbon hydroxylase, but not by allylisopropylacetamide or indomethacin, inhibitors of drug inducible cytochrome P450 or prostagladin cyclooxygenase, respectively. The lack of toxicity of 3-methylocholanthrene toward late passage rat epidermal cells can be attributed to the low constitutive rate of biotransformation these cells exhibit. The insensitivity of rat esophageal cells despite substantial metabolic activity reflects the importance of intrinsic differences among keratinocytes derived from different epithelia and species in determining toxic response. Human cervical and monkey esophageal keratinocyte cultures also actively metabolized benzo(a)pyrene, illustrating further the utility of the culture system for exploring differences among epithelial cell types.

Epidermal growth factor (EGF) and the tumor-promoting diterpene phorbol 12-myristate-13-acetate (PMA) have been shown previously to increase the production of prostaglandin E_2 (PGE_2) in bone, a biochemical action which leads to resorption of extracellular matrix and calcium release. In this report, we have identified specific, high-affinity receptors for EGF and phorbol esters on 4 lines of PGE_2-producing human osteosarcoma cells; G-292, TX-4, MG-63, and SaOS-2. EGF receptors in all 4 lines had similar affinities and binding capacities, and exhibited homologous down-modulation after incubation with EGF. In addition, each cell line showed specific high affinity binding of [^{3}H]phorbol 12,13-dibutyrate. The lines differed, however, in their biological responses to EGF and PMA.

Respiratory Biology and Inhalation Toxicology:

This program is under the direction of Dr. Joseph D. Brain. The research and graduate training activities are primarily concerned with the lung as a major interface between environmental chemicals and the body. Topics of interest include factors influencing uptake, distribution, retention and fate of inspired particles; types of response to toxic particles; bioassay methods, role of alveolar macrophages in defense responses of the lungs, and others. The ultimate goal is better understanding of the role of environmental factors in the etiology of acute and chronic diseases of the lung.

During the past two years, a hamster model of interstitial pulmonary fibrosis has been developed which mimics the human lesion. The effects of the combined drug and oxidant injury was studied in hamsters sacrificed at 3, 6, 10, 30, 60, 90 and 120 days after 0.16 μ bleomycin instillation on the basis of histopathologic and morphometric assessment. At 3 days, an early exudative interstitial pneumonitis was seen, which became proliferative at 6 and 10 days. By 30 days, the intense acute cellular infiltrate had subsided with a remnant focal interstitial pneumonitis and fibrosis involving 10.5±6.2% of the lung. With time, the fibrosis involved increasing amounts of lung. By 120 days, the lungs showed an interstitial pneumonitis with a diffuse network of delicate fibrosis, involving 38.8±14.4% of the lung. Hamsters treated with 0.16 μ bleomycin alone, saline with 70% oxygen for 72 hours, or saline alone showed no significant pathology at 30 days through 120 days. This significant increase in disease when the treatments are combined demonstrates the potential for even moderate levels of hyperoxia to enhance the pulmonary fibrotic effects of bleomycin at levels where fibrosis would not be expected.

Among several approaches being planned for trial in pilot studies, an organ culture system has been developed that appears promising as a model for studying the functions of solitary pulmonary endocrine cells and neuroepithelial bodies, insofar as

these cells differentiate in the pulmonary epithelium after the cultures are explanted. These can be observed in semi-thin plastic sections stained by any of several methods selective for neuroendocrine cells. Because the cultures are small (1-2 mm in diameter), the neuroepithelial bodies are easily located for ultrastructural study as well. In culture, these bodies occur in the lining of a sac (the dilated bronchial tree) that is sealed off from the outside, a condition favoring use of the cultures to investigate endocrine cell reactions to various pharmacological agents that might be introduced into the culture medium or into the sac. The cultures could prove as useful for isolating neuroepithelial cell responses made in the absence of connections to the central nervous system from those made with connections intact. Development of the cultures proceeds according to a timetable similar to that followed in vivo.

An in vivo bioassay to predict the pulmonary toxicity of particulates, gases, and other agents using biochemical and cellular indicators of damage in lung lavage fluid has been developed: a) in situ phagocytic ability of the pulmonary macrophages as measured by the lambda bioassay of Brain and Corkery; b) inflammatory response as shown by increases in macrophage and polymorphonuclear netrophil (PMN) numbers; c) damage to the air-blood barrier as indicated by increases in serum albumin, d) direct cellular effects demonstrated by the release of lactate dehydrogenase (LDH), a cytoplasmic enzyme, and beta-N-acetyl-glucosaminidase, and peroxidase, which are both lysosomal enzymes, and e) proteolytic activity using a synthetic substrate for the neutral protease elastase. These parameters reflect different early manifestations of lung injury which can have long term pathological consequences.

Epidemiologic Studies:

The key faculty members in the research reported here are Dr. Richard Monson (Program Director for Environmental Epidemiology and Occupational Health), Dr. Thomas Smith (Director of the Environmental Assessment Program), and Dr. Benjamin Ferris, Jr. (Principal Investigator of the Six Cities Study of Air Pollution).

The epidemiologic studies program has several components. One is concerned primarily with health effects which may be associated with factors in the work environment. The adverse health effects of greatest interest in the past have been respiratory diseases and cancer; recently interest has been growing in possible effects of occupational exposure on the nervous system or on the reproductive system. In addition, opportunities are seized when they present themselves to investigate problems that may relate to any biologic system. Another component of epidemiologic studies puts less emphasis on field observations depending instead on analysis of mortality experience in relation to a range of environmental variables which may include work histories, chronic disease treatment regimens, place of residence, and many other factors.

Particularly interesting results have developed during the past year in the study of B.F. Goodrich rubber workers. Analyses were conducted on the mortality experience of groups of employees with specific work experience. Among workers who make rubber, two groups were evaluated. Persons exposed to the raw materials that are used in preparing rubber had an excess of stomach or intestine cancer (33 observed, 17.7 expected). Persons exposed to green rubber and to solvents had an excess of leukemia (14 observed, 7.3 expected). Also, among 327 persons

who had potential exposure to acrylonitrile there were nine deaths from lung cancer observed and 5.9 expected. In another study of over 12,000 pregnancies in Boston, analyses were collected of the associations of adverse outcome of pregnancy with maternal consumption of cigarettes and coffee. Cigarette smoking, but not coffee drinking, was associated with low birth weight. No association between coffee drinking and congenital malformation was noted.

The other component of the program is study of the effects of community air pollution, including indoor pollution, on the respiratory systems of children and adults. These studies and the occupational field studies have much in common; both must include intensive efforts to characterize the environment and to assess the quantity of actual human exposure; both must take into account and try to control for important confounding variables such as tobacco smoking.

The Six-Cities Study represents a multidisciplinary prospective investigation of the effects of outdoor and indoor air pollutants on pulmonary function and respiratory disease during childhood and adult life. Enrollment of 13,853 children and 8,859 adults began in six eastern and midwestern cities between 1974 and 1977, accompanied by an extensive air quality monitoring program of both outdoor and indoor environments. The children have been seen annually and the adults every three years. The data are currently complete through the 1980-81 examination and are being analyzed. Preliminary results initially indicated that gas stoves had a significant effect on children's health. Over time this has persisted qualitatively, but has become not statistically significant. Maternal smoking, however, has shown an effect on children's pulmonary function (reduction) and an increase in respiratory symptoms.

University of Cincinnati

The research and teaching staff of the center encompasses many basic science and clinical disciplines as well as levels of interest. The capabilities represented by the department staff include: analytical, organic, physical and biological chemistry, biostatistics; cell biology; chemical, environmental, mechanical and safety engineering; cutaneous biology and dermatology; history of science; immunology; internal medicine; meterology; microbiology; neurochemistry and neurophysiology; nutrition; oncology; pathology; pediatrics; pharmacology; physics; renal physiology; pulmonary physiology and chest diseases; psychology; psychiatry; radiologic health; and toxicology. Programs are designed so that each component project can benefit from close interaction, assistance from, and coordination with other core research units or subunits.

Previously it was reported that cholesterol exacerbates the effects of cadmium on δ-aminolevulnic acid dehydratase (ALAD) and high density lipoprotein (HDL)-cholesterol in weanling Sprague-Dawley rats (1). Work was continued to determine the effects of cadmium and cholesterol on the distribution of zinc, copper, iron, and cadmium in tissues of exposed rats as well as on the alteration of liver lipids. The findings suggest that the simultaneous exposure of rats to cadmium and cholesterol exerts an effect on lipid metabolism. Together with the previously reported alterations in the distribution of cholesterol in serum lipoprotein, there is a significant modifying effect of cadmium on liver lipids. It can be further deduced that the presence of cholesterol does not alter the tissue accumulation of cadmium, indicating that the synthesis of metallothionein remains unaltered. These results confirm a pronounced effect of cadmium on lipid

metabolism. This effect is accompanied by significant changes in essential metal metabolism in tissues.

Epidemiologic investigations for identifying workers with asthma involves a multidisciplinary approach utilizing respiratory questionnaire data, pulmonary function testing, and immunologic investigations. An additionally important test for confirming asthma is the demonstration of airways hyperreactivity, perhaps an early manifestation of disease. Tests measuring nonspecific bronchial hyperreactivity, such as exercise or cold air challenge, have shown good correlation with more traditional tests using pharmacologic agents like histamine and methacholine. Airway hyperreactivity usually accompanies occupational asthma and may be an early indicator of disease. Surveillance programs utilizing cold air challenge provide rapid and acceptable procedures for detection. Cold air bronchial challenge testing is useful for population studies to identify airways hyperreactivity and may identify early cases of asthma.

Epidemiological evidence from studies on As-exposed populations strongly suggest that As is carcinogenic and, presumably, a gene toxin. Unsuccessful attempts to induce cancer in laboratory animals by exposure to As seem to contradict the conclusions from epidemiological evidence. However, Nishioka (3) reported positive mutagenic responses to As by E. coli and Nakamuro and Sayato and Wan et al. demonstrated increased chromosomal aberrations and sister chromatid exchange with tri- and pentavalent As in human leucocytes and Chinese hamster ovary cells, respectively. Rossman et al. dispute Nishioka's findings, since they were unable to show a mutagenic response from arsenite treatment of E. coli or Chinese hamster (V79) cells. Rossman argues that arsenite acts as a cocarcinogen; he demonstrated an enhancement of recovery of uv-induced E. coli mutants by treatment with arsenite. Studies on the comutagenic properties of arsenite in V79 cells were undertaken to confirm the findings of Rossman et al. The results indicate that arsenite is not mutagenic when cultures are exposed to the concentration or exposure period used by Rossman et al. However, at more toxic concentrations, or after longer exposure periods, arsenite is mutagenic.

A study has been made of the effects of arsenite ($NaAsO_2$) and arsenate (Na_2HAsO_4) on CHO cells and lymphocytes. It was found that inhibition of CHO cell growth was directly related to the dose of $NaAsO_2$. The 50% growth inhibition dose for CHO cells was calculated to be 8×10^{-9} M.

Chromosome analysis of CHO cells treated with $NaAsO_2$ and Na_2HAsO_4 show chromosome aberrations in all categories (with the possible exception of fragments, which were rare). This was best illustrated in the category of chromosome breaks. However, two (or more) hit aberrations, i.e., rings, exchanges, and multiple breaks, also showed a dose-response relationship. Taken together, this latter group made up the majority of aberrations at all dose levels except the lowest dose of 1×10^{-6} M $NaAsO_2$. Arc sine transformed percentages of aberrant metaphases were regressed against concentrations of arsenical. Linear regression analysis of the data showed a significant relationship ($p \leq .001$) between concentration and occurrence of aberrant metaphases. The trivalent arsenical $NaAsO_2$ was 5-10 times more effective than the pentavalent arsenical, Na_2HAsO_4.

The effect of $NaAsO_2$ on stimulated human lymphocytes in four separate experiments was significantly related to the dose of arsenite. The lowest dose tested which caused chromosome damage in human lymphocytes was 0.5×10^{-6} $NaAsO_2$, whereas in CHO it was 1×10^{-6} M.

Analysis of SCE in CHO cells indicates that sodium arsenite caused a dose-related increase in SCE in cultured CHO cells in the same dose range that produces chromosome breakage. Results show that both sodium arsenite and sodium arsenate are effective clastogens in the dose range studied. Furthermore, increases in SCE in CHO cells, which are also considered to be an indicator of mutagenesis, were related to doses of arsenite.

Fecal extracts from many normal individuals contain mutagen(s) that can be assayed with Salmonella tester strains (Bruce et al., 1977). There are significant differences in the prevalence of active donors in different populations; the values are higher in populations on Western diets than are observed for those on Black South African, vegetarian or Japanese diets. These observations have suggested that mutagen levels might be affected by dietary factors. In experiments conducted this year, it was found that the supplemental ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) depressed fecal mutagenicity in 20 healthy human donors aged 22 to 55 years. The vitamins were given at a dose of 400 mg daily each. The mutagen(s) was extracted from feces samples with dichloromethane, and assayed with Salmonella typhimurium tester strain TA-100 without microsomal activation. The addition of Vitamin C and E to the diet of one male donor on a highly controlled diet decreased the yield of fecal mutagenic activity to 21% of the initial control period. In the 19 subjects participating in the second study, 8 did not produce detectable amounts of the mutagen. The activity in the other 11 donors dropped on the average to 26% of the control period value during the second two week period when they received a daily dose of 400 mg of Vitamin C and Vitamin E. Adding Vitamin C and Vitamin E directly to the feces did not affect mutagen activity.

A procedure has been developed for the preparation of active fecal mutagen samples employing anaerobic incubation at 37° for 4 days. The mutagen is extracted with an organic solvent, selectively absorbed and eluted from a silica Sep-Pak and quantitated using HPLC with UV and florescence detectors. Addition of some human and baboon bile samples before incubation produced slight increases in mutagen yield but the results were not sufficiently impressive to recommend their routine use. Addition of ^{14}C acetate and propionate to the incubations may have led to the formation of labeled mutagen but the small amounts of mutagen in the purified extracts compared to the amounts of inactive and probably labeled contaminants made it impossible to demonstrate labeling of the mutagen unequivocally.

Studies were initiated on the effects of tumor promoters on B cell mitogenesis. Treatment of spleen cell suspensions with the specific B cell activator E. Coli. lipopolysaccaride (LPS) led to a mitogenic response, as measured by cell counting or uptake of tritiated thymidine, with a characteristic dose dependence. Similar treatment with the potent tumor-promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA), or its solvent, at the appropriate concentration was without mitogenic effect. TPA and LPS were present simultaneously, however, an increased mitogenic response above that of the activator alone was observed at LPS concentrations below 100 µg/ml. This concentration was found optimal in experiments involving the latter agent alone. The comitogenic activity of TPA was dose-dependent, and comitogenicity was found to a lesser degree with other tumor-promoting phorbol diesters. The nonpromoting analog phorbol was found inactive, however.

Although the immunosuppressive properties of carrageenan (Cg) are well documented, the mechanism by which this agent exerts its immunosuppressive effect is not clear.

The relative loss of immune responsiveness resulting from Cg treatment may be explained by the ability of macrophages, or their secretory products, to suppress lymphocyte responsiveness. Results from the present study support the hypothesis that Cg-induced immunosuppression may be mediated by suppressor cells belonging to an adherent cell population. The observation that peritoneal macrophages from Cg-treated rats were actively secreting an inhibitory factor as determined in vitro provides evidence that in vitro and in vivo Cg-induced mitogenic suppression may share a common effector mechanism. Despite the long and widespread use of Cg as a food additive, this is the first evidence of its immunosuppressive potential following ingestion.

An unusual pattern of cell death and limb malformations has been observed in mice treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The optimum dose of MNNG for induction of limb malformations was found to be 50 mg/kg. This dose induced death in 1.4% of the litter, limb malformations in 63% of the survivors, and a significant depression in fetal body weight. Gross inspection indicated that hindlimbs were more frequently malformed than forelimbs, and limbs on the left side deformed more frequently than on the right. No abnormalities were observed in the long bones of control limbs, but reduced metacarpal #4 in forelimbs, and metatarsal #1 in hindlimbs occurred. In treated fetuses, the long bones were shortened more frequently in LFL than in RFL, as was ectrodactyly of digits 4 and 5 or absence of metacarpals 3 and 4. Shortening of the femur and postaxial reduction and twisting of the fibula occurred more frequently in LHL and RHL. Essentially the same pattern of malformation was observed in both hindlimbs, but both long bone and metatarsal abnormalities were more frequent in LHL than RHL.

Oregon State University

During this past year research has been pursued on a variety of chemicals to which humans may be exposed. Among these are the polychlorinated biphenyls, 2,4,5-T, pentachlorophenol and its contaminants such as the chlorophenoxyphenols, methylmercury, halogenated hydrocarbons, aflatoxins and pyrrolizidine alkaloids. The types of studies included determination of the biological effects on whole organisms, tissues and cells, biochemical mechanisms of action and the dynamics of the chemicals in organisms and the environment. In addition, mathematical modeling of the dynamics of chemicals and development of new statistical techniques for treating toxicological data are being investigated. Among the biological effects studied include those on membranes of cells, immunological responses and carcinogenesis.

A study was made of binding of polychlorinated biphenyls and other toxicants in human cells grown in culture. PCBs bind to the cell surface and are taken up by skin fibroblasts. Most of the bound PCBs are absorbed. Microscopic fluorescence single cell analysis showed that cytoplasmic inclusions are found early after treatment. The inclusions contain phospholipids and glycerides. The PCB-induced lipidosis in skin fibroblasts was reversed by high density lipoprotein and partially by LDL, but not by very low density lipoprotein (VLDL). Subcellular fractionation showed that 95% of the HCB is bound to the supernatant fraction, presumably by the endoplasmic reticulum and associated enzymes.

The effects of 2,4,5-T on the immunocompetence of mice was investigated. Male mice exposed to 2,4,5-T for 6 weeks showed reductions in both numbers of circulating white blood cells and numbers of nucleated cells present in the spleen. Female mice also exhibited reduced splenic cellularity after 6 weeks of exposure. These changes were not evident in either male or female mice exposed to 2,4,5-T for 12 weeks. T cell blastogenesis (Con A, PHA) was enhanced in male mice exposed to 2,4,5-T for 6 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. A detailed statistical analysis of the blastogensis data has not been completed at this time. 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 (0.088ppm) in the 2,4,5-T was sufficiently below the level necessary to cause immunotoxicity.

A study was conducted on the effect of methylmercury on transplacental tumor induction with nitrite and ethylurea. 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. Progeny from all strains developed 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 MeHg dose-response experiment. The highest levels 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 of incidence and latency of neural tumor development is in progress.

The basic properties of glutathione conjugates were studied. Glutathione half-mustard formation appears to occur during the metabolism of vicinal dihalo aliphatic hydrocarbons, e.g. 1,2-dichloro- and 1,2-dibromoethane. The rates of hydrolysis of related S-(2-haloethyl)- cysteine analogs was investigated by titrimetrically measuring the rate of sodium hydroxide addition required to maintain pH, as the halo group of the compound was lost and replaced by a hydroxyl group. S-(2-hydroxyethyl)-cysteine and respective hydrolysis products were identified using HPLC. The half-life of S-(2-chloroethyl)-cysteine at pH 6.1, 37°, was 11 min., while that of S-(2-bromoethyl)-cysteine was 5 min. (pH 6.1, 37°). The hydrolysis rate was dependent upon concentration of analog, temperature and pH of the reaction, suggesting a different mechanism than that seen for β-chloroamines. The charge on the compound was a significant factor in the pH dependence of the reaction. These studies indicate a non-classical S_N1 mechanism of hydrolysis for these sulfur mustards, with further investigation needed to probe the participation of neighboring groups in these reactions.

Basic studies with model membrane systems were conducted during the past year. Spacings and side chain conformations of hydrated multilayer vesicles of BrDPPC over the temperature interval -40 to 80°C were determined. The major part of the work involved sample temperatures above -10°C where water is in a supercooled or normal state. It was shown that the presence of a single bromine atom, randomly substitute at the C_9 or C_{10} position of the α chain of DPPC, is sufficient to broaden or destroy the well known solid-liquid side chain transition which occurs

at 41°C in DPPC. These results are consistent with earlier x-ray studies of oriented samples and with calorimetric results obtained with BrDPPC dispersions in ethylene glycol. Samples were 50-50 weight percent lipid-water mixtures. Thus the lipid is fully hydrated and is representative of biological conditions. The main conclusion is that bromination at the midpoint of the acyl side chain greatly enhances membrane fluidity. This would have implications for membrane functions, such as membrane protein orientation, diffusion or phase separation, which are dependent on lipid states. These might be affected by incorporation of significant amounts of brominated fatty acids present as food additives into biological lipids.

Several of the scientists at OSU have observed a phenomenon that could have far-reaching implications in human diseases and environmental pollution. This involves chemical induction of virus production in uninfected chick embryo cells. They have a research program to examine pyrrolizidine alkaloid structure to determine the structural components necessary for inducing the 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. During this grant period they were able to demonstrate that jacobine, a major alkaloid found in tansy ragwort, was capable of inducing virus synthesis in uninfected cells. They have modified the chemical structure of jacobine and have shown that certain modified chemical derivatives will induce virus synthesis, while other modifications of the structure render it inactive. The importance of these findings is that chemical structure affecting the expression of cellular genes can be altered to change this biological activity. In the long term it is hoped that this study will be able 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. This will be important for evaluating the structural properties of chemicals in the environment having potential to modify disease expression.

The effect of technical pentacholorophenol exposure on the humoral immune response has been examined. Exposure levels to PCP ranged from 50 to 500 ppm PCP in the diet for 8 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 response were significantly suppressed. In parallel with the reduced anti-DNP 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 in injection of cyclophosphamide. A direct effect of PCP on the B cell is suggested by these findings.

New York University

The hypothesis that nutrition has a large effect on cancer occurrence in populations throughout the world is being investigated. 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 inhibitor, is being studied. It has been found to occur in canned legumes and in tofu. One inhibitor from tofu, the Bowman-Birk inhibito

has been purified; it is heat stable and is not destroyed by pepsin. This laboratory found that the Bowman-Birk and chick-pea inhibitors are more active in inhibiting superoxide production by neutrophiles than is the Kunitz soybean inhibitor. Thus, these smaller inhibitors have the stability to survive cooking procedures used in food preparation, are not destroyed by gastric digestion and are effective in blocking an essential action of the tumor promoter.

Investigations were extended on the development of atherosclerotic lesions in experimental animals (cockerels) exposed to carcinogens in vivo. In short-term exposure studies it was found that a single injection of 7,12-dimethylbenz(a)anthracene (DMBA) to four-week old cockerels resulted in an increase in lesion cell proliferation at five weeks, compared to controls. This proliferation increase preceded an increase in lesion size. The atherogenic response to DMBA was not limited to younger animals. Birds receiving weekly DMBA injections from forty to fifty weeks of age displayed lesions whose median size was similar to that of twenty-week old animals exposed to DMBA weekly from four to twenty weeks of age. Lesion size distributions and labeling indices were similar in the two groups. Roosters sixty-five weeks of age and older also responded to DMBA treatment with a marked increase in lesion size.

Ultrastructural studies provided strong support for earlier conclusions that in this system carcinogens exerted their effect upon pre-existing lesions. Spontaneous and carcinogen-associated lesions were virtually indistinguishable via transmission electron microscopy.

Studies have been made of changes occurring in mouse epidermal nonhistone nuclear proteins (NHNP) following application of initiating and promoting agents to mouse skin. It was, therefore, essential to obtain pure mouse epidermal nuclei. Final purification of the nuclei was achieved by banding in metrizamide density gradients. Studies on NHNP thus far reveal marked differences in NHNP from mouse epidermal nuclei, mouse nuclei and mouse sarcoma nuclei.

Studies have been initiated on the mechanism(s) of mutagenicity and carcinogenicity of acrylonitrile. Acrylonitrile has been reacted with the nucleosides found in DNA, namely 2'-deoxycytidine, 2'-deoxyguanosine and thymidine at 37°C and pH 7.0-7.5. For each reaction, new adducts have been detected by analytical paper chromatography suggesting that acrylonitrile may be capable of functioning as a direct-acting carcinogen because it readily formed new DNA base adducts.

Studies have been made of the genetic toxicology of carcinogenic metals. Chromate seems to be the metal which behaves most like a classical carcinogen in that it is both mutagenic and causes the induction of λ prophase (the inductest). Its mutagenicity can be most easily demonstrated in the fluctuation test. In order to assess the role of DNA repair in chromate mutagenesis, a variety of strains defective in one or more DNA repair pathways were compared. Results show that mutagenesis by chromate is unaffected by the urvA-dependent excision repair pathway and is SOS-independent. This supports the view that chromate causes 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. The comutagenesis effect does not occur with arsenate.

Hydrogen chloride (HCl) and formaldehyde (HCHO) are both irritating compounds which can chemically react to form bis(chloromethyl)ether, a human and animal carcinogen. Preliminary studies have shown a significant carcinogenic effect in rat nasal epithelia when they were exposed to an atmosphere of HCl and HCHO. The present ongoing study was started to find out 1) whether the combined exposure is necessary to produce cancer, 2) whether either HCHO alone or HCl is carcinogenic, 3) if the combined exposure is necessary for carcinogenic response, and would the response be the same if the HCl and HCHO are introduced into the inhalation chamber separately.

Throughout the experiment, the concentrations of HCHO and HCl were very close to the target-levels of 14 ppm and 10 ppm, respectively. Tumors were found only in the nasal cavity in all the treated groups except in animals receiving HCl alone. There were 21 tumors in the combined premixed group, 7 in the combined non-premixed group, 18 in the HCHO group and none in the HCl group. The preliminary results suggest that HCHO is the responsible agent in all three groups for the induction of nasal tumors.

The effects of day versus night exposure to acrylonitrile in young Sprague-Dawley rats is being investigated. One group was exposed to acrylonitrile at 150 ppm for 4 hours between 6 AM and 10 AM. Another group was exposed between 6 PM and 10 PM. There were marked differences in weight gain as a result of time of day of exposure. Differences in metabolism were generally minimal suggesting that enzyme induction had not occurred as a result of multiple exposure, whether day or night. However, as expected, glutathione was depressed as a result of acrylonitrile exposure and differences were noted between acute and sub-chronic as well as male versus female and day versus night exposure. The latter findings are significant because the time chosen for most inhalation toxicity studies are the times of lowest animal activity, least mixed function oxidase activity and highest glutathione concentration.

About 2,700 children given X-ray therapy for thymic enlargement and their 5,000 siblings have been followed. Analysis of data from a new survey of these groups has just begun. The thyroid doses ranged from 5 to over 1,000 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 group of about 2,200 persons given X-ray therapy for ringworm of the scalp (tinea capitis) for up to 35 years ago and a control group of about 1,400 patients who did not receive X-ray treatment have been followed. The irradiated group has shown an excess of benign thyroid tumors, as compared to the controls, even though the thyroid dose was only about 6 rads. However, no thyroid cancers have been observed to date. An excess of brain tumors and skin cancers has also been observed in the irradiated group. A comparison of the locations of the skin cancers with the X-ray dosimetry of the skin suggest that x-irradiation and ultraviolet exposure potentiate each other.

Data acquisition has been completed in a study of the effects of childhood lead poisoning on later neuropsychological 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.

Research is continuing on the rates and products of photodegradation of particle-bound polycyclic aromatic hydrocarbons (PAH) under simulated atmospheric conditions. The degradation of PAH has been found to follow an apparent first order rate law in the presence of air. In experiments with coal fly ash as the particle substrate, the order of stability of three PAH compounds was benzo(e)-pyrene > pyrene > benzo(ghi)perylene, which parallels the stability predicted by Huckel molecular orbital theory. The same order of stability was observed in experiments on the degradation of PAH on coal fly ash as a settled dust. In these latter experiments, however, the half-lives of the order of hours were observed. Rates and products of degradation of pyrene on carbon and on glass have also been investigated using the photoreactor. The half-lives on glass, fly ash and pure carbon were 1.0, 7.5, and 31 hours, respectively. The principal product of degradation for the carbon substrate was 1,1-bipyrene. No evidence of quinones or quinols has been observed to date for the carbon substrate although these are formed with the glass. Compounds eluting at the same retention times as fluor-anthene and benzo(e)pyrene were observed when the carbon was used, suggesting that rearrangement reactions occurred.

Preliminary discussions have been held aimed at a collaborative investigation with scientists in the People's Republic of China, where about 1,500 cases of lung cancer have occurred in a tin mining community in Yunnan Province. There is also a high incidence of arsenic poisoning. Most of the cases occur among the tin miners, but members of the general community have been affected as well.

The circumstances are complex, involving exposure to radon and arsenic as well as to other mineral dusts, tobacco smoke, and, in case of the general community, sulfur dioxide. Standard mortality ratios for lung cancer are somewhat higher than for other cohorts of miners, including those in Eastern Europe and Southwest United States. Fumes from the smelter have contaminated nearby water supplies and farming areas. Detailed discussions of possible areas of collaboration were held in China during October, 1981, and joint research projects are being developed.

Vanderbilt University

Somatic cell hybrids were made between mouse myeloma cells and spleen cells derived from Balb/c mice immunized with liver microsomal cytochrome P-450 purified from rats treated with 3-methylcholanthrene (MC-P-450). Thirty-seven independent hybrid clones among 66 tested produced monoclonal antibodies to the MC-P-450 as measured by radioimmunoassay. Six of the hybridoma clones were chosen for further study. Five produced IgG1 in culture and the sixth clone produced IgM. The monoclonal antibodies formed were positive for MC-P-450 in respect to protein binding measured by radioimmunoassay, precipitation of the enzyme and inhibition

of enzymatic activity. Analysis by gel electrophoresis indicated that a single microsomal protein band interacted with the antibody and this band co-migrated with MC-P-450. These monoclonal antibodies also interacted with the major form of cytochrome P-450 from β-naphthoflavone-induced rats as well as with MC-P-450 but did not bind, precipitate, or inhibit the activity of the major form of P-450 from phenobarbital-treated rats. The monoclonal antibodies inhibited benzo(a)-pyrene hydroxylation activity of the purified MC-P-450 by more than 90 percent, as measured by the aryl hydrocarbon hydroxylase assay for phenol production. Analyses of benzo(a)pyrene metabolism by high pressure liquid chromatography indicate that the monoclonal antibodies inhibited the enzyme activity of the purified MC-P-450 for all of the positions at which oxidation occurs. The monoclonal antibodies also inhibited both aryl hydrocarbon hydroxylase and 7-ethoxy-coumarin deethylase of liver microsomes from 3-methylcholanthrene-treated rats by 70 percent, indicating that these activites are functions of an enzyme(s) equally affected by antibody binding to a common or identical antigenic site which represents at least 70 percent of the total activity in these microsomes. Microsomes from control or phenobarbital-treated rats were unaffected, suggesting that this antigenic site on the MC-P-450 is absent in the microsomes from control or phenobarbital-treated rats. High pressure liquid chromatographic analysis of antibody inhibition of benzo(a)pyrene metabolism by microsomes from 3-methyl-cholanthrene-treated rats showed an inhibition of phenol and diol formation that ranged from 46-72 percent. 1,6-Quinone production was not affected which suggests that this metabolite was formed by another cytochrome P-450 isozyme or nonezymatically. The specificity of monoclonal antibodies will be useful in the study of substrate and inducer specificity, and in the identification and assay of mutliple forms of the cytochrome P-450 and the determination of their content and function in different tissues, species and individuals.

The distribution of microsomal flavin-containing monoxygenase (N,N-dimethylaniline N-oxidizing) was examined using a technique involving separation of proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, electrophoretic transfer to nitrocellulose sheets, staining of the sheets for the enzyme by sequential treatment with rabbit anti-porcine liver monooxygenase, goat anti-rabbit immunoglobulin G, and horseradish peroxidase/rabbit anti-peroxidase complex and densitometry of the visualized bands. Because of the lack of immunochemical identity of the enzyme among the various species examined and the unavailability of purified enzyme from species other than hog, only relative estimates could be made for the levels of enzyme found in the various tissues examined. Enzyme levels were estimated in the liver, lung, and kidney of the rat and mouse and the liver and lung of rabbit. In all cases the highest enzyme concentration was found in liver. However, in the male (but not female) mouse the enzyme concentration was nearly as high in lung and kidney as in liver. These observations are all consonant with previous reports of the distribution of N,N-dimethylaniline oxidase activity. However, the lability of enzyme activity and the presence of potential endogenous substrates and effectors are not factors in estimates of enzyme distribution made with this technique. The qualitative and quantitative data obtained using these immunochemical techniques may be of further use in understanding the role of this enzyme in various processes involving both endogenous and xenobiotic compounds.

Epoxide hydrolase was purified to electrophoretic homogeneity from human liver cytosol using hydrolytic activity towards trans-8-ethylstyrene-7,8,-oxide (TESO) as an assay. The overall purification was 400-fold. The purified enzyme has an apparent monomeric molecular weight of 58000, significantly greater than the

50000 found for human (or rat) liver microsomal epoxide hydrolase, or for another TESO-hydrolyzing enzyme also isolated from human liver cytosol. Purified cytosolic TESO hydrolase catalyzes the hydrolysis of cis-8-ethylstyrene-7,8-oxide ten times more rapidly than does the microsomal enzyme, catalyzes the hydrolysis of TESO and trans-stilbene oxide as rapidly as the microsomal enzyme, but catalyzes the hydrolysis of styrene-7,8-oxide, p-nitrostyrene-7,8-oxide, and naphthalene-1,2-oxide much less effectively than does the microsomal enzyme. Purified cytosolic TESO hydrolase does not hydrolyze benzo(a)pyrene-4,5-oxide, a substrate for the microsomal enzyme. The activities of the purified enzymes can explain the specific activities observed with subcellular fractions. Anti-human liver microsomal epoxide hydrolase did not recognize cytosolic TESO hydrolase in purified form or in cytosol, as judged by double-diffusion immunoprecipitin analysis, precipitation of enzymatic activity, and immunoelectrophoretic techniques. Cytosolic TESO hydrolase and microsomal epoxide hydrolase were also distinguished by peptide mapping. The results provide evidence that physically different forms of epoxide hydrolase exist in different subcellular fractions and can have markedly different substrate specificities.

Specific immunochemical techniques were used to quantitate the levels of eight isozymes of cytochrome P-450 (P-450) and epoxide hydrolase in liver microsomes of untreated rats and rats treated with phenobarbital, 3-methylcholanthrene, a mixture of these two compounds, nine individual polybrominated biphenyl (PBB) congeners, and a commercial mixture of PBB's.

Eight different forms of cytochrome P-450 (P-450) were purified to electrophoretic homogeneity by a common procedure from liver microsomes of rats treated with phenobarbital of β-naphthoflavone. Antibodies were prepared to seven of these forms in rabbits. The eight P-450s were distinguished by spectral properties of the ferric, ferrous, and ferrous carbonyl forms, apparent monomeric molecular weights, peptide mapping, immunological reactivity as discerned by double-diffusion immunoprecipitin analysis and crossed immunoelectrophoresis, and catalytic activities toward the substrates acetanilide, aminopyrine, aniline, benzo(a)pyrene, d-benzphetamine, N,N-dimethynitrosamine, 7-ethoxycoumarin, 7-ethoxyresorufin, ethylmorphine, p-nitroanisole, testosterone, and R- and S-warfarin.

In an attempt to identify and characterize specific protein-DNA complexes, the protein-DNA cross-linking by UV and gamma irradiation as well as BCNU and cis-Pt was investigated. Two antisera were employed in the experiments. Both gamma and UV irradiation, exposure to cis-Pt and, to a lesser extent, BCNU, resulted in cross-linking of a select group of antigens to the DNA. Although several antigens were cross-linked by all the employed agents, others exhibited agent-specific cross-linking patterns. Moreover, there were distinct differences between cross-linked proteins detectable by the two antisera.

Another experimental series involved studies on the protein-DNA cross-linking by various salts of chromium and nickel. Using the above described approach trivalent chromium cross-linked a number of chromosomal nonhistone protein antigens to the DNA. The cross-linking increased both with incubation time and concentration. Nickel was considerably less effective, although the same family of antigenic proteins was found cross-linked as in the chromium experiments. Preliminary investigations indicate that the same proteins become salts in tissue cultures. Characterization of the cross-linked proteins is being attempted.

In another project, chromosomal protein antigens in rat hepatomas were studied. Earlier findings indicated that highly specific antisera can be elicited by injecting rabbits with preparations of dehistonized chromatin. Such antisera recognized both the confirmational specificity of chromosomal nonhistone protein complexes with DNA and individual protein antigens.

In cadmium antidote studies it was found that mice which have been given the cadium equivalent of $CdCl_2.2\frac{1}{2}H_2O$ at a level of 10 mg/kg, diethyldithiocarbamate (DDTC) administration at 500 mg/kg have a higher survival rate than is found with Na_2Ca-EDTA under identical conditions. This occurs when the interval between administration of the cadmium salt and the antidote is either one or two hours. The surviving animals, however, are found to have appreciable levels of cadium in the brain, liver and kidney when they were treated with DDTC, though their behavior appeared to be normal. The use of 50 mg/kg of DDTC after 2 hours in such cadmium poisoned mice also leads to a higher survival rate and reduced amounts of cadmium retained in the brain, liver and kidney. Unlike the other chelating agents which have been reported as useful in acute cadmium intoxication, DDTC appears to act by forming a lipid soluble complex which is largely immobilized in lipid containing tissues of the animal's body.

Studies on antidotes for beryllium and uranium have shown that ortho diphenols and organic phosphonates of the correct structural types are effective antidotes for both Be^{++} and UO_2^{++} intoxication. Tiron (sodium 4,5-dihydroxybenzene-1,3,disulfonate) is very effective in enhancing the urinary excretion of both of these elements from acutely intoxicated rats.

In continuing efforts to identify the causative agent(s) of equine leucoencephalomalacia (ELEM) and pulmonary edema of swine (SPE), two metabolites formed by Fusarium moniliforme growing on toxic corn samples were characterized with regard to toxicity for animals and other biological properties. The first of these, 4-ethyguaiacol, has been detected in a wide variety of sources. It exhibits low toxicity for laboratory rodents and equines but has significant antimicrobial properties for several pathogenic bacteria and dermatophytic fungi. The second metabolite, which also has antimicrobial activity, was eventually identified as fusaric acid. Toxicity tests in laboratory animals and equines failed to attribute a role for this toxin in ELEM, although it is very acutely toxic to rodents. Difficulty has been experienced in obtaining large quantities of ELEM toxic corn to serve as a reservoir for both large and small animal feeding trials. No success was otained in demonstrating toxicity of Fusarium moniliforme-infected corn for BD-IX rats, chicks, or day-old peking ducklings.

Initial studies on the pulmonary toxicity of perilla ketone (PK) for chronically instrumented, unanesthetized sheep have produced some enlightening information indicating this purple mint toxin differs in its lung edemagenic properties from several other toxic agents or models. PK administration increased lung lymph flow without remarkable hemodynamic effects. The increased flow coincided with increases in the lymph to plasma protein concentration ratios, pointing to increased capillary permeability. The lymph of intoxicated sheep also contained increased levels of archidonic acid metabolites, viz. TxB_2 and 6-keto PGF. The latter metabolite increased to higher levels and remained significantly elevated throughout a 5 hour observation period. Whether prostacyclin release contributed to the pathogenesis of acute respiratory failure due to PK administration or occurs in response to lung injury is not as yet clear.

The effects of vinyl chloride-polyvinyl chloride exposure on the respiratory system of exposed workers seem to indicate two patterns of nonmalignant effects: a granulomatous reaction to PVC dust and an interstitial pulmonary fibrosis due to vinyl chloride monomer effect on protein molecules which trigger immunologic responses to the altered protein. The long-term carcinogenic effect with a significant increase in the incidence of lung cancer is a matter of concern as well, although the magnitude of this effect has not yet been completely evaluated.

The hepatic lesions associated with vinyl chloride in man and rodents exhibit an identical sequence--from proliferation of hepatocytes and sinusoidal cells, frequently associated with sinusoidal dilatations, to angiosarcoma. Often, at least in young rodents, hepatocellular carcinoma is also observed. The demonstrated similarity of the evolution in man and rodents strongly supports the extrapolation of observations from experimental animals to man.

An increased prevalence of abnormal nerve conduction velocities (NCV) was found among chemical workers exposed to the phenoxy herbicides 2,4,5-T and 2,4-D. The NCV of the median motor and sural sensory were significantly slower in the study versus control group. Slowed sural sensory velocity was significantly correlated with duration of employment. Since this group of workers was exposed to both 2,4,-D and 2,4,5-T, as well as their associated chlorinated dioxin contaminants, the new slowing may have been influenced by any or all of these substances. This possibility is being further investigated by NCV studies of another group of workers who has been exposed to 2,4,5-T (contaminated with dioxin) but not exposed to 2,4,-D.

Among 171 farm residents exposed to PBB, serum PBB values decreased over a four-year period. The estimated change of approximately 25% incorporates a wide range (0-90 ppb) of values and an inherent error of ±10%. A continued decline of this magnitude would suggest a clearance time of 60-70 years for p,p'-DDE and PBB. The value for DDE is comparable to that reported elsewhere in the literature.

Successful transplantation of human malignant mesothelioma has been achieved. The success rate was high (65% of mice and 64% of implants) and tumors grew readily. The availability of this model can now open the way to the study of chemotherapeutic sensitivity of these tumors, with practical consequences for clincal trials. Since the mean delay of appearance and growth of tumors is 46 days, much shorter than that for mesotheliomas caused by injection of asbestos fibers into rodents, this should allow the determination of chemosensitivity of a tumor in real time, with potential usefulness for individual patients.

An assessment of occupational asbestos exposure in the United States between 1940 and 1979 shows that the present population at risk is very large, approximately 27,500,000 individuals. This figure does not include the number of individuals exposed to asbestos in family contact circumstances, although this number too may be substantial. Nor have estimates been included of asbestos exposure in the Armed Services although many individuals may have had such exposure, particularly in the Navy. Approximately 8,200 asbestos-related excess cancer deaths are currently occurring annually. This will rise to about 9,700 annually by the year 2000. Thereafter, the mortality rate from past exposures will decrease, but still remain significant for another three decades.

A study of 17,800 asbestos insulation workers, followed for a ten-year period, has provided quantitative information on the period of latency between onset of exposure to asbestos and death, including cancer death. There was little increase in either cancer deaths or of asbestosis observed in less than 15-19 years from onset of exposure. After 20, 30 or 40 years, however, the number of cancer deaths began to be of significance. From these data, it appears that it would be advantageous to analyze the experience of exposed individuals in duration-from-onset exposure categories of more than 30 years. There is the risk of obscuring the neoplastic effects of asbestos exposure by the co-mingling of later deaths with those that may have occurred much earlier and could be much less likely to be influenced by asbestos exposure. Unless the later years are followed, only the very limited early effects will be identified and the full import of the exposures may not be appreciated.

While the mechanism of action of lead in various organ systems is not completely understood, experimental studies have demonstrated disturbed porphyrin metabolism in the central nervous system as a result of lead exposure. Although extrapolation from animal cell culture to humans is uncertain in this regard, it can be concluded that for chronically exposed individuals, the ZPP level seems to be a better guide than the blood lead for evaluating several health-related lead effects. The test is rapid and inexpensive, and if used as a primary screening parameter, it will make the surveying of large communities and occupational groups more effective. It was found that quantitative measures of oculomotor performance were correlated with blood lead and zinc protoporphyrin (ZPP) levels. Both the total number of eye movements and the saccades-to-target correlate significantly with blood lead and ZPP levels. In addition, the slope of amplitude-velocity relationship correlated significantly with ZPP levels. These results add further support to evidence that ZPP levels are an excellent indicator of chronic effects of low-level lead exposure.

It was found that the risk of renal function impairment increases with higher lead exposures and absorption. ZPP levels appear to be a more clear-cut indicator than blood lead levels for differentiating between occupational groups with different levels of chronic lead exposure. Based on this comparative study, a level of blood lead and of ZPP can be defined above which there is a definite risk for renal function impairment with long-term exposure: for blood lead, levels in excess of 60 mg/100ml and for ZPP, levels in excess of 100 mg/100ml.

It was thought that the hydrogen bonding character of quartz surface was primarily implicated in the pathogenesis of silicosis. However, it has been found that inhibition of hemolysis can occur by bonding of a cationic metal as well. The two types of inhibitors, the hydrogen bonding polymer and the metal ions, have been shown to be independent of one another and non-interfering. Which of the blocked functionalities (or both) is responsible for membrane damage remains unanswered. The surface of quartz seems clearly bifunctional, the ratio of sites dependent on the degree of ionization of the silanol groups. The contribution of the negative surface charge to the hemolytic activity of the quartz appears to be real, significant, and of sizable magnitude.

Two natural zeolite minerals, erionite, a fibrous form, and mordenite, a mixture of fibrous and granular types, were injected intraperitoneally into 45 Swiss albino male mice with a single administration of 10 mgs or 30 mgs suspended in 1 cc of saline solution. Six of ten mice treated with 10 mgs of erionite developed malignant peritoneal tumors between eight and 22 months after the sing

administration. The neoplasms in four of the six animals were consistent with malignant mesothelioma. Two of four chrysotile treated controls also developed malignant peritoneal mesotheliomas between seven and 16 months. A fibrogenic effect was noted in both the erionite and mordenite-treated mice, the effect being more pronounced in the former.

Asbestos has been established as the primary cause of most cases of diffuse malignant mesothelioma occurring in the industrialized world; animal experimentation has supported these epidemiologic conclusions. This study, using a non-asbestos-containing zeolite mineral (erionite) adds new evidence to the phenomenon of fiber carcinogenesis. The findings suggest that the carcinogenic and fibrogenic effects of the fibrous erionite are similar to those of asbestos.

Two recent studies have indicated that low levels of "passive" smoking increase risk of lung cancer to a significant degree. Apart from the practical issue that this provides evidence that cigarette smokers might be increasing the risk of lung cancer in their fellow citizens, the data may be used to illuminate the portion of the dose-response curve for very low doses, and to gain insight into the shape of the dose-response curve at low doses. Analyses suggest that the dose-response curve at very low doses is approximately logistic in shape (flattened S shaped) rather than a straight line. If this is so for one chemical carcinogen (tobacco smoke) and for one type of cancer, it is likely to be so for other chemical carcinogens and for other types of cancer. This could radically alter present impressions of "tolerable levels of exposure" for a host of other agents.

A large-scale clinical field study has been mounted of the Mohawk Indians who live at St. Regis, and concomitantly, a number of Mohawk Indians who reside in another Indian Reserve at Caughnawaga. This Reserve is also on the St. Lawrence River about 80 miles east of St. Regis. The residents are closely allied with the St. Regis Indians genetically, culturally, socially and economically, but are not known to have had fluorine or mercury exposure. By comparing the two populations, it is anticipated that information will be obtained about potential health effects that might be associated with several environmental contaminants, including fluorides, mercury, polychlorinated biphenyls and Mirex.

There is a large literature on the impact of high levels of fluorine on plants, animals and especially cattle and in humans on tooth enamel and dental caries, on bone development, immunological changes, alterations in liver function tests, thyroid and other endocrinological abnormalities and neurological changes. However, there is little comparable information on the effect of low level fluoride exposures.

This study hopefully will yield information on the health status of Mohawk Indians who have been exposed to low level ambient fluoride contamination, as compared with Mohawk Indians who live in comparable areas without such contamination. In addition, it is expected that the data will have public health implications for many other communities with fluoridation programs to prevent dental caries. The results of this large-scale clinical field study should provide baseline information on the overall health effects of low level fluoride exposure.

A large population in Iraq was accidentally exposed to a methyl mercury compound in the winter of 1971-1972. The continuing study of the aftermath of this outbreak is concentrating on establishing quantitative relationships between prenatal exposure and postnatal effects. Studies have revealed a relationship between the number of abnormal neurological signs in children of pre-school age and the maximum concentration of mercury in their mother's head hair during pregnancy. The increase in the number of abnormal signs above spontaneous background becomes statistically significant at maternal hair concentrations below 125 ppm and above 13 ppm. Signs of severe motor retardation were seen in five children for whom maternal peak hair values were between 165 and 320 ppm.

A second group of children have been studied who experienced exposure to methyl mercury from the mother's milk. Some of these also received additional exposure from ingestion of contaminated bread during the suckling period. Despite high blood levels in the first few months of life, the infants were remarkable for the mildness of the clinical manifestations. The main findings were hyperflexia and delayed achievement of developmental milestones.

Following earlier observations in the Iraq outbreak that adult females might be more sensitive than males to neurological damage from methyl mercury, tests on rats were carried out to check this possibility. Female rats, treated daily by gastric gavage with 8.0 mg Hg/kg as methyl mercury, developed more intensive coordination disorders and had more extensive damage in the granular layer of the cerebellum than similarly treated male rats.

Follow-up metabolic and clinical studies of infants exposed to a phenyl mercury compound in their diapers are still at an early stage. The median urine concentration in the first sample collected from over 200 infants was 106 μg Hg/l was found. Samples of head hair contained phenyl mercury identified by gas chromatography and it accounted for about 80 percent of the total mercury, the remainder being in the inorganic form. Urine samples contained mainly inorganic mercury following cessation of use of the contaminated diapers.

A few cases of acrodynia were found and a number of non-specific signs and symptoms were recorded - anorexia, asthenia, dermatitis, diarrhea, hypotonia, insomnia, irritability, nervousness and profuse sweating. In general, the prevalence of these non-specific effects was low and it is not clear if mercury was the causative factor. Studies are continuing, including observations on a control group of infants.

Sensory systems, because of their special vulnerability, have continued to be a prominent theme in the research program at Rochester. The visual system has been investigated from several different functional standpoints, including both psychophysical and electrophysical methods, and with a close interweaving of function and histopathy. Acrylamide is an important lesson because now it appears that an episode of moderate intoxication may leave permanent residual losses of visual acuity. Tactual sensitivity seems to recover completely, but requires several months to do so. Sensory testing is typically carried out in primates, and current data point to a peculiar recovery phenomenon, a type of resistance, following methyl mercury poisoning to a level of mild incapacitation. Whether this phenomenon is expressed histopathologically is being examined. Any molecular basis is speculative, but the molecular mechanism of methyl mercury

toxicity is being carefully dissected, beginning with the processes by which rat brain muscarinic receptors are inhibited by methyl mercury. It has now been shown that D-penicillamine can reactivate the muscarinic receptor.

Lead is another toxic heavy metal whose actions are still a puzzle. Behavioral studies since the late 1960s have featured a model based on exposure of rodents through maternal milk. Yet, the human problems arise from exposure later in life (including adulthood). It has now been demonstrated that the juvenile rodent is also quite susceptible to lead toxicity (perhaps even more than the neonate). The choice of a suitable behavioral endpoint is critical, however.

Solvents and fuels pose significant problems for environmental health science because of their wide distribution as both industrial chemical and consumer products. A comprehensive behavioral toxicology of these substances is essential for setting standards. The effects of toluene on operant behavior, for example, mimic those of the minor tranquilizers and barbiturates, providing a clue to toluene abuse. Methanol, known as a potent poison, is a possible replacement for petroleum-based fuels, but almost nothing is known about its low-level, chronic toxicity. It now appears to be a behavioral teratogen in rodents under these conditions.

Air pollutants and irritants are not generally included in surveys of behavioral toxicology, but behavioral techniques can help assess properties such as irritant potency. Ozone, for example, reduces the output of operant behavior in rats, and, at levels equivalent to Clean Air Act limits, running activity.

During this past year studies were made of effects of alteration in species and numbers of gut microflora resulting from dietary changes as young mice are weaned from a suckled milk diet to solid pellet food intake, and as adult mice are changed from a solid pellet diet to milk alone or to a high protein, low fat liquid diet. Large changes were observed in rates of demethylation of methyl mercury and large changes in rates of whole body mercury elimination associated with these diet related changes in gut microflora. Antibiotic treatment to eliminate intestinal flora also markedly reduced demethylation of methyl mercury and fecal mercury elimination after methyl mercury administration. These results indicate that gut microflora play a significant role in demethylating methyl mercury and thereby increasing fecal mercury excretion.

In vitro metabolism of methyl mercury was also studied by incubating radioactive methyl mercury with cecal or small intestinal contents and determining rates and extents of demethylation. Significant demethylation of methyl mercury has been observed *in vitro* and is currently being determined the chemical nature of the resultant mercury compounds. Preliminary results indicate that microflora changes with human weaning from mild intake are also associated with increased rates of demethylation.

The effect of the naturally occurring glucocorticoid cortisol (hydrocortisone) on renal mercury binding was examined using the renal mercury sexual dimorphism mouse model system previously described. Treatment of adult males with cortisol causes a sharp, dose-dependent reduction in kidney mercury concentration to levels similar to those observed in females. Cortisol treatment significantly reduces mercury body burdens of both male and female methyl mercury treated mice. These observations may provide insight for designing treatment strategies.

Studies have been conducted to determine whether exposure to methylmercuric chloride during mid to late fetal stages causes reproductive toxicity in adulthood, potential target sites being the neuroendocrine system or germ cells. Although adult body weight showed a significant dose-related decrease, reproductive performance (litter size) was only slightly decreased (in females); these effects resulted from exposure at fetal ages of 12.5 and 15.5 (but not 9.5) days post-fertilization. However, neither postnatal growth nor reproductive performance was as sensitive to prenatal methyl mercury as was rate of survival at birth.

Studies on the metabolism of mercury vapor have concentrated on the rate of oxidation of elemental (Hg^0) to ionic (Hg^{++}). Since previous studies have shown that Hg^0 dissolved in plasma is probably the predominant mobile species of mercury after inhalation of mercury vapor, the rate of removal of Hg^0 by oxidation is a critical process affecting the mobility of mercury in the body.

A method has been developed to measure dissolved vapor in blood and to preserve the blood sample in such a way as to maintain the concentration of dissolved vapor unchanged. The results indicate that oxidation in the red blood cells follows "zero order" kinetics, i.e. is independent of the concentration of dissolved vapor in blood.

Studies of metallothionein (MT) induction and MT effects on the metabolism of toxicity of metals are continuing. A sensitive radioimmunoassay has been developed for measuring MT in rat plasma and in rat and human urine. Elevated levels of MT in rat urine have been found after injection of Cd, Hg, Cu, and Zn, but not Pb. Human studies of MT levels in urine from Japanese women living in Cd-polluted areas and from workers exposed to Cd, indicated that elevated MT is associated with other urinary abnormalities, eg., proteinuria.

In vitro, rat hepatocytes have been investigated in relation to MT synthesis. Study of sexual dimorphism in the metabolism of Hg vapor has been undertaken in several strains of mice and found related to enhanced Hg excretion by females: this can be modulated by testosterone administration. Other sexual differences in Hg metabolism and tissue binding are under investigation.

The lymphatic uptake of ^{109}Cd from the lungs of dogs was investigated after aerosol exposure, with and without prior exposures to CdO aerosol. The results showed that the lymphatic uptake of Cd is not normally an important alveolar clearance pathway; however, chronic, pre-exposures to CdO significantly increased (factor of 10) the subsequent lymphatic uptake of ^{109}Cd, apparently due to enhanced alveolar permeation. This viewpoint was substantiated by the use of ^{99m}Tc-DTPA aerosol which also showed a markedly enhanced uptake (reduced lung retention half-time) after chronic CdO exposures. Other aspects of intravenous and pulmonary administrations of Cd were investigated, including efforts to apply chronically-implanted lymphatic cannulae.

In acutely-exposed rats and dogs, fiber particles (UICC amosite) are found in lung lymphatics and the cells of the pleural space are responsive. Possible mechanisms are under study in rats. A relationship has been found between the complex [^3H]estradiol binding mechanism of the estrogen receptor and the conformation changes induced by ligand-binding that are associated with the transformation of the receptor from nonactivated into an activated state. The activated state of the receptor interacts with the nucleus and increases RNA synthesis. Insight into the relationship of the [^3H]estradiol-binding kinetics and receptor

activation has led to the development of a new method of analysis that measures the effects of estrogenic xenobiotics (kepone, DDT and clomiphene) upon receptor activation.

The assay for the 2,3,6,8-tetrachlorodibenzo-p-dioxin (TCDD) receptor has been greatly improved using hydroxylapatite to separate the receptor [^{3}H]TCDD complex from the unbound [^{3}H]TCDD. This method has allowed a more accurate measure of the receptor's affinity and receptor concentration among various tissues and species. The relationship between the receptor's molecular properties and the toxicity of TCDD in various species was explored. The tissue distribution, excretion and metabolism of [^{3}H]TCDD in various responsive and nonresponsive mice has been investigated. The mechanism of the TCDD induced hyperlipidemia in the guinea pig has been initiated.

Insight of the dynamic equilibrium between the various DNA structures, i.e., β-DNA and beta kinked DNA has also been greatly improved. These studies of drug-DNA interaction provide an understanding of the RNA transcription, recognition sites and the mechanisms of drug-induced mutagenesis and carcinogenesis.

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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 these active forms with the test bacteria, two tester strains were developed 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. Such a model was developed that works quite well for this whole class of compounds. An enzyme preparation, which is called fecalase, is made by sonicating human feces (which is made up of bacteria to a large extent). Fecalase contains a wide variety of enzymes that split sugars from glycosides. By adding fecalase to the Salmonella test, it was shown that many different naturally occurring glycosides of mutagens (flavonoids, anthraquinones, cycasin, etc.) show up as mutagens.

A method was developed to examine oxidative DNA damage in humans by examining deoxynucleoside bases in human urine. This work has led to the discovery of a major anti-oxidant in human blood that is not present at appreciable levels in rats and mice and may have been important in the evolution of a long lifespan in primates.

The ions of certain heavy metals are well known to be mutagenic and carcinogenic, but as yet very little is understood about the means whereby these ions enter the cell and contact the DNA. This process is being investigated in bacteria, with a focus on iron complexed in the form of low molecular weight carriers termed siderophores. The outer membrane protein which serves as a receptor for the specific siderophore ferric enterobactin has been shown to possess phenylalanine at the N-terminus. Following proteolysis by protein a, the new N-terminus was identified as alanine. These data show that protein a acts as an endopeptidase and may indicate that the active site of the receptor is near the amino end of the 81,000 MW polypeptide. The receptor has been isolated from the outer membrane in an apparently native state and its dissociation constant with the ligand measured as about 3×10^{-8} M. One of the mutations affecting transport of another specific siderophore, ferrichrome, has been found to be required for utilization of yet other siderophores, including those specified by plasmids. A specific transport system was detected in E. coli for the uptake of ferric rhodotorulate, a siderophore from yeast species. These studies are beginning to disclose the genetics and biochemistry of iron assimilation in enteric bacteria. This system is believed to be a suitable model for uptake of both nutritious and noxious metal ions in microorgainsms, plants and animals.

Mutagenic agents can exert their effects directly on DNA nucleotides or they can cause mutations by inducing a process known as error-prone or "SOS" DNA repair. Recent studies have shown that this process may be mediated by an altered form of DNA polymerase which is observed in cells only after induction of the error-prone synthesis. The nature of the alteration in this enzyme and measuring the frequency with which it makes errors in the replication of defined template DNAs is being studied.

Three major new DNA repair pathways have been characterized. Two involve new DNA repair endonucleases of E. coli, endonuclease III and endonuclease V. The former appears to recognize particular types of damage in products brought about by radiation or chemicals; the latter appears to recognize damage in the DNA by virtue of distortion and/or uracil content. The third, and perhaps most unusual, involves the joint action of endonuclease VI and endonuclease III to remove from DNA deoxyribose phosphate present at apurinic or apyrimidinic sites.

In addition, several human repair enzymes were characterized, 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 -- an important finding. Finally, it was shown that, as is the case in E. coli, human cells have two ATP endonucleases that can act together to excise dexoyribose phosphate from AP DNA.

Mutagenesis and teratogenesis may occur by defects in mitotic mechanisms that control the separation of sister chromatids during cell division. Work is continuing 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 hydrolyzed during

mitotic assembly. Kinetic studies show that ATP enhances the nucleation of assembly and the elongation as well. The other major advance this year concerned the synthesis and turnover of H1 histone variants, and in particular $H1^0$, which is associated with the arrest of mitosis. When mitosis is inhibited, $H1^0$ and certain other H1 subtypes are synthesized in preference to other H1's, and there is differential breakdown among the various H1's. As a result, the pattern of H1's and $H1^0$ changes over a period of a few days, as cell cultures cease cell division. Two other structural proteins (HMG1 and 2) also responded to mitotic inhibition, but only when cells became committed to differentitation.
An understanding of the structural effects of changing patterns of H1's and HMG1 and 2 could help to explain the mechanics of sister chromatid separation and gene control. Knowledge of microtubule and spindle formation is also essential for understanding chromatin separations, both normal and aberrant.

A study is being made of the eukaryotic cell surface and its assembly. Techniques were developed 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 mutant 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 maturation steps, and a pathway of secretory organelles.

A study 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.

The comparison of the pattern of accumulation of mutations in silent and functional ζ globin genes is leading to thinking about the influence of chromosomal domains on gene regulation and the acceptability of mutations.

Massachusetts Institute of Technology

It was found that the biologically active compounds in combustion exhausts can be produced by mechanisms independent of the formation of soot particulates. This result is important because abatement strategies and environmental regulations are aimed at reducing particulates. It is not possible to characterize combustion conditions within real operating ranges which emit very low amounts of soot but high amounts of polycyclic aromatic hydrocarbons (PAH).

Secondly, it was found that, in the several combustion processes studied, the PAH are responsible for the biological activity measured (genetic mutations of bacteria and human cells). Other mutagens, such as formaldehyde, are emitted in fair quantities; but, when one considers biological potency and amounts produced together, the PAH and their polar derivatives are the primary actors in laboratory test systems.

Thirdly, compounds of the PAH class were found which were previously unsuspected as mutagens are really responsible for large fractions of the biological activity of exhausts. Furthermore, fluoranthene, 1-methylphenanthrene and cyclopenta-(c,d)pyrene were found equally mutagenic in human and bacterial cells with benzo(a)pyrene, but chemical analysis found them to be present in large excess over this compound, B(a)P, which is apparently falsely accorded the status of the most important PAH air pollutant.

Finally, and most important to the future contributions, it is now feasible to separate the conditions of combustion chemistry into three operational categories:

a. Fuel and oxygen are well mixed and react above ignition temperature;
b. Fuel is heated in the near absence of oxygen; and
c. Fuel and oxygen react at temperatures below ignition.

Combinations of these conditions exist in practical combustion systems--learning to separate these variables and measure the chemical products associated with each--gives a most useful tool for understanding and reducing the biological consequences of combustion for energy production.

Development of accurate and reproducible techniques and tests for production, identification and biological assay of combustion-produced mutagens is an important aspect of this work during the first four years.

The polycyclic aromatic, fluoranthene, has emerged as a major mutagen. It is as active as benzo(a)pyrene in both bacterial and human lymphoblast assays and is a major component of samples from laboratory flames and from practical combustion systems. While it was not reported to cause tumors in published mouse skin painting tests, exploratory work with newborn mice has demonstrated its ability cause lung adenomas. Some of its principal metabolites have been identified and its principal DNA adduct isolated. Cyclopenta(cd)pyrene is also an important component of combustion--produced PAH and is active in both human and in bacterial cell tests. Fluoranthene appears in significant quantities in all combustion systems--cyclopenta(cd)pyrene is significant when well-mixed flames above ignition temperatures produce the major amounts of PAH. Benzo(a)pyrene, while active, is of less importance because of its generally low concentration. Methyl phenanthrenes and other alkylated PAH are important mutagens in samples from diesel engines and other combustion systems where opportunities exist for reactions below ignition temperature (pyrolysis).

Samples taken from the residential oil burner, the diesel engine and gas turbine show substantial mutational activity in bacterial tests without the mammalian enzyme activation that is required for demonstration of activity by simple PAH. While aldehyde substituted PAH and other polar compounds are active as "direct-acting" mutagens and are found in large quantities from combustors like diesels, all of this type of activity cannot be accounted for by summing the activities of individual compounds.

Well-defined laboratory flames, burning pre-vaporized and pre-mixed fuels, produce the PAH, such as fluoranthene and cyclopenta(c,d)pyrene, but do not produce "direct-acting" polar mutagens. It, therefore, appears that these polar compounds are formed in accompanying non-flame processes--such as oxidative pyrolysis, which results from mixing fuel with hot combustion products under conditions where reactions without ignition occur. As noted above, such processes are believed t

occur in the turbulent diffusion flames employed in residential oil burners, diesel engines, furnaces and gas turbines.

Separating the variables of combustion has been achieved to a large extent by building laboratory combustors in which one form of combustion condition predominates. A laminar flat flame burner and well-stirred reactor, where an attempt is made to eliminate composition and temperature gradients in the combustion zone, have been employed to study the formation of PAH in toluene, heptane and ethylene-air flames. This work indicates that PAH, and probably soot, grow by addition of C_2 and C_4 fuel fragments to aromatic molecules. PAH formation is extremely sensitive to temperature. For example, pyrene production, in a well-stirred reactor, is reduced from 50 µg/g toluene to 2 µg/g, when reaction temperature is increased from 1580 C to 1670 C. As fuel/air ratio is increased, PAH production increases; and, at a critical ratio of fuel/air, soot appears. As more fuel is added, PAH production decreases and production of soot increases very rapidly. It appears that synthesis of soot consumes part of the reactive intermediates that would otherwise form PAH. The laminar flat flame burner produces soot at a lower ratio of fuel to air and, within the flame zone, produces very large concentrations of PAH which are partly consumed in the downstream portion of the flame. It is believed that quenching of this flame zone may be responsible for production of PAH in gasoline engines. By using intense mixing or high combustion temperatures, production of PAH and soot can be greatly reduced.

Not all fuels, however, are readily dispersed in a gas phase that is "well-mixed" at a desired temperature. When coal or heavy fuel oils are burned, they produce gas phase tars that subsequently burn. Defects in the flame will allow these vapors to escape. These tars contain large concentrations of PAH. This process was studied under well-defined conditions in a special "drop tube furnace" and also in the large experimental furnace. It was found that the production of PAH goes through a maximum as temperature is increased, but that soot formation increases continuously with temperature. At very high temperatures, all the tar and PAH can be converted to soot and gas demonstrating that, under such conditions (partial pyrolysis in diffusion flames), soot production does not necessarily correlate with PAH. Similarly, in the experimental furnace, large amounts of soot and PAH are produced near the burner inlet, while the ratio of soot to PAH decreases drastically as these materials are burned out.

Studies of the residential oil burner have shown that this device normally produces much less PAH than the automotive diesel engine for the same fuel consumption. However, in residential areas during winter, much more fuel is burned for space heating than for transportation, and exposure to PAH from this source would seem to be important. Test conditions mimicking home use, i.e., cyclic operation, produced about ten times the PAH found in continuous operation and changed the chemical profile of the exhaust in significant ways. In these burners, soot production is controlled by adding the necessary amount of excess air. In some cases, it was found that this smoke control strategy increased PAH production.

Duke University Marine Laboratory

Studies are being carried out on the accessability to nucleases of chromatin that is complexed with different histone variants. These studies make use of the sea urchin Lytechinis variegatus as a model system. During sea urchin embryogenesis, stage-specific changes in histones H(1), H(11A), and H(11B) occur concommitantly with changes in chromatin structure. The fact that both the nucleic acids and the proteins associated with developing organisms are subject to various environmental perturbants make these studies using a marine-organism model particularly worthwhile. The feasibility of producing antibodies specific for the individual histone variants found in sea urchin embryos is also being studied. These antibodies will facilitate studies on the effects of histone variants on the structure and function of chromatin and the roles which these variants play in differentiation. The investigations include the general objective of understanding the mechanisms for control of gene activity and differentiation and the influences of environmental stresses (e.g. pollutants) on these processes.

Studies are continuing on the toxicity of oxygen and on the protective mechanisms which make aerobic life possible. Oxygen toxicity is largely due to reactive intermediates generated by reduction. These include the superoxide radical, hydrogen peroxide and the hydroxyl radical. The defenses include superoxide dismutases, which catalytically scavenge the superoxide radical, catalases and peroxidases, which eliminate hydrogen peroxide, and antioxidants which minimize the damage done by the hydroxyl radical. Circumstances which lead to increased production of these intermediates of oxygen reduction, or which diminish the capacity of the defenses, result in damage or death of the cells.

P. leiognathi lives as a symbiont in a specially evolved gland in the abdomen of the ponyfish. The daytime luminescence of the bacterial symbiont on the ventral surface of the fish allows it to nearly match the luminosity of the surface waters and thus to be camouflaged. This bacterial symbiont is peculiar in that it contains a copper/zinc-superoxide dismutase in addition to an iron-superoxide dismutase. Iron-superoxide dismutases are commonly found in prokaryotes, but the copper/zinc enzymes are not. It had been supposed that this could reflect a gene transfer from the fish to the bacterium. Data strongly supporting this natural gene transfer from a eukaryote to a prokaryote have now been obtained.

Organisms often respond to increased levels of threat by raising the appropriate defenses. Thus, increasing the rate of production of O_2 often elicits increased biosynthesis of superoxide dismutase. It was noted that elevating superoxide dismutase in the unicellular alga Chlorella provides increased resistance not only to O_2 , but also to sulfur dioxide. A hypothesis has been advanced to explain this cross-protection and it is being further examined.

Lactobacillus plantarum cannot synthesize heme but can incorporate heme into catalase. Some strains of this bacterium continue to make catalase in the absence of heme. This peculiar catalase has been called pseudocatalase to distinguish it from the familiar heme-containing catalase. Pseudocatalase has been isolated and found to be a new manganese-containing enzyme. The manganese at the active site seems to cycle between the Mn(III) and Mn(V) states during catalysis. This is the first enzyme in which this cycle of valence states has been encountered. The pseudocatalase is extremely useful in the practical sense since it is not poisone

by azide, cyanide, sulfide or mercurials and is very stable to freezing and thawing.

The structure of bovine copper-zinc superoxide dismutase (SOD) has been determined to 2Å resolution. Atomic coordinates were refined using a computer program for stereochemically restrained refinement against structure fractures. The bovine copper-zinc SOD is a dimer, each enzyme subunit composed primarily of 8 anti-parallel beta strands that form a flattened cylinder, plus 3 external loops. The beta strands form a beta barrel structure which is assymetrical and can be viewed as having two distinct sides; beta strands 5 to 8 are shorter with fewer hydrogen bonds, less regular side chain alternation and greater twists than strands 1 to 4. The active site consists of copper (II) and zinc (II) which lie 6.3 Å apart at the bottom of a long channel in the protein. The zinc is buried while the copper is solvent-accessible.

Work has continued on analysis of red blood cells from marine and terrestrial organisms as possible targets for organic and inorganic pollutants. The studies have also included oxygen-carrying proteins which occur outside red blood cells - hemerythrins (non-heme oxygen-carrying proteins with binuclear iron centers), and hemocyanins (binuclear copper-based proteins), and the giant extracellular hemoglobins. An investigation of the interaction of various lead compounds with human hemoglobin and red blood cells has shown that this important metallic pollutant has marked effects on human hemoglobin function.

Respiratory proteins involved in electron transport have also been worked on. The membrane-bound mitochondrial protein, cytochrome c oxidase, has been investigated in terms of its interaction with various forms of mercury. Mercuric chloride as well as organomercurials have been found to bind specifically to Subunit II of the cytochrome c oxidase system. This metal-protein interaction leads to significant impairment of function that is evident under steady-state turnover conditions but not under those for the enzyme in its "pulsed" or "resting" states. The implied differences in the conformational states of the enzyme may be clarified by further studies of the structural and functional consequences of mercury binding.

The interaction of hexane metabolites on hemoglobin function is being investigated. 2-5-Hexanedione acts as a protein crosslinking reagent. This compound has been used to investigate the delayed neurotoxicity of hexane metabolites. It was found that rats intoxicated with this compound appeared to be cyanotic. Investigations are underway to determine whether this cyanosis is due to a direct interaction of the chemical with rat red cells and/or hemoglobin. As in the case with the hexanedione-exposed rats, cyanosis is noted in chickens exposed to n-Butyl mercaptan and there is a definite inducement in methemoglobin formation in chickens exposed to this compound.

A Feasibility Study is being carried out on the interaction of 3,4-dimethyl-2,5-hexane dione (DMHD) with the seaworm Myxicola infundibulum. It was found that DMHD is 16 times more toxic than 2,5-HD on a molar basis and, as 2,5-HD does, forms pyrroles and crosslinks proteins. The marine worm Myxicola has a simple nervous system from which pure axoplasm is readily obtained from the unmyelinated nerve cord. Hence, pure neurofilament preparations can be isolated in a single centrifugation. The experiments done with the Myxicola are critical to the project in the sense that they will test in vivo both whether the neurofilaments are covalently crosslinked and whether 2,5-HD and DMHD are bound to neurofilaments in

this process. Myxicola is offering a unique opportunity to answer these questions because of its simple nervous system, the absence of myelin and the ease with which neurofilaments can be isolated. Further, in comparison with rodents, smaller quantities of labeled compounds will be required and higher exposure levels tolerated.

University of Washington Marine Center

Toxaphene, a chlorinated camphene insecticide, is a hepatic carcinogen in rodents and an aquatic pollutant which 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.

The in vitro 50% growth inhibition for toxaphene-exposed cells was considerably higher than the in vivo LC50 value 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 able to convert 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, i.e. the trabecular type, which consists of broad trabeculae 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, especia the presence of aberrant bile canaliculi, may represent preneoplastic stages and warrant further study with a large sample size.

A number of studies have demonstrated that the bile of fishes exposed to certain xenobiotics contains metabolites at concentrations as high as 10,000 times the concentration in the water. The large majority of these compounds are present in bile as biotransformation products of the parent compound, generally as glucuronide conjugates.

Recent studies have demonstrated that bile may serve as a source for mutagenic metabolites. Significant mutagenic activity in bile of rats administered 2-acetylaminofluorene, benzo(a)pyrene, 2-aminoanthracene, 5-amino chrysene and other carcinogenic compounds has been detected for the salmonella histidine reversion mutagenicity assay (Ames test) in the absence of the S-9 microsomal activation fraction in some instances, indicating that activated carcinogens are excreted directly into bile of rats. Equally important was the finding that addition of β-glucuronidase to the medium greatly augmented the mutagenic activity of bile from carcinogen-treated rats. Because of the concentrating and storage function of the trout gallbladder, the bile of trout should provide an excellent source for estimating the presence of mutagens (carcinogens) in the aquatic environment, and thus may provide a valuable tool for assessing long-range health effects (mutagensis and carcinogenesis) of water pollution.

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 from 1×10^{-6} to 2×10^{-4}. Exposure of this cell line to B(A)P resulted in a significant, but much less dramatic, increase in mutant frequency. Verification of the resistance levels of mutant clones to OUA showed an increase in resistance from 10^{-7} M to 10^{-4} M. Prolonged growth of the cells in non-selective medium did not diminish their level of resistance to OUA. Ouabain was used as a selective agent because BF cells appear to be lacking a functional HGPRT system.

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.

The effects of several metabolic inhibitors are being studied on the phenotypic expression of certain genes in a purified differentiating cell line isolated from sea urchin embryos. This work has two major goals: 1) elucidation of the mechanisms of control of gene expression in development. The inhibitors block known links in the chain of biochemical events of normal gene expression. They are, therefore, useful tools for determining the levels of control of expression of individual genes during development. 2) to establish the sea urchin primary mesenchyme cell line as a useful system for the detection and study of potentially harmful environmental agents that act by altering the normal program of gene expression in developing embryos.

AIR POLLUTANTS AND RESPIRATORY DISEASE

A study on the effects of ozone (O_3) on airways is being carried out in sheep. Antigen-induced bronchoconstriction was not enhanced 24 hours after exposure to O_3 in sheep, but the severity of the bronchospasm was reduced if the sheep inhaled antigen only two hours after being exposed to O_3. Data from studies of breathing patterns during O_3 exposure, indicate that the latter finding is the result of prior release of histamine from mast cells by the O_3. Finally, the severity of bacterial pneumonia is enhanced in sheep having received a bacterial inoculation prior to O_3 exposure. These findings suggest that O_3 can produce alterations in normal airways and thus may pose a risk to individuals with airways which are already compromised by disease.

Structural effects of alkyl nitrites on the oxidation of oxyhemoglobin and deoxyhemoglobin have been determined using a representative series of structurally varient alkyl nitrites. The composite results are consistent with an inner sphere electron transfer mechanism for oxidation that is the first model of such a transformation for hemoproteins. This inner sphere oxidation methodology can now be used to probe accessibility to the heme site of hemoproteins. Initial investigations of the effects of ionic surfactants on hemoglobin have demonstrated significant influences on hemoglobin structure and reactivity. These influences, which occur at surfactant concentrations equivalent to those of hemoglobin, appear to be associated with the hemoglobin dimer-tetramer equilibrium.

Respiratory anaphylaxis to industrial chemicals may be manifest in several ways. Immediate-onset, delayed-onset, and hypersensitivity pneumonitis have all been recognized following exposure of sensitized workers to airborne industrial chemicals. During the past year, progress has been made in development of animal models for each of the three respiratory hypersensitivity reactions. For immediate sensitivity, guinea-pigs were exposed to known concentrations of toluene diisocyanate (TDI) vapor for 3 hrs. per day on 5 consecutive days. Three weeks later, animals were evaluated for dermal sensitivity, circulating homocytotrophic antibody titer and pulmonary sensitivity. Results indicated that pulmonary sensitivity and antibody production were dependent upon the concentration of inhaled TDI. Delayed-onset pulmonary sensitivity was experimentally produced in animals by injection with Freund's complete adjuvant. Bronchial provocation challenge with tuberculin protein elicited pulmonary responses with maximum reaction 9-12 hrs. following challenge. Delayed reactions were characterized by mononuclear cell infiltration into pulmonary tissue. An animal model for hypersensitivity pneumonitis was developed using Naegleria gruberi. The above animal models should enable comparison of chemicals for pulmonary sensitizing potency.

Conditions for the production of nitrite and N_2O by an obligate methanotroph, Methylosinus trichosporium were studied. Both methane and oxygen were required for the production of nitrite from ammonia oxidation. N_2O production was

positively correlated with the amount of produced nitrite and with very low level of oxygen. The production of N_2O (10 uM) was, however, much less than the disappearance of nitrite (100 uM) when oxygen was below 0.2 mM. It is not clear at this moment if the unaccounted loss of large amounts of nitrite was due to further reduction to hydroxylamine and/or ammonia. The nitrite and N_2O production rates by Methylosinus trichosporium were 6.9×10^{-3} and 2.2×10^{-14} mmol d^{-1} $cell^{-1}$, respectively. These values are about 0.2% and 2.2% of the highest reported values for Nitrosomonas europaea. Considering the average time of cell division of methane oxidizers is shorter than that of chemoautotrophic nitrifiers and that the former can reach approximately 200 times higher cell densities, the rates of both nitrite and N_2O production by methane oxidizers may be as significant as those of Nitrosomonas in aquatic environments.

Rats with chronic pulmonary infections secondary to intratracheal injections of Pseudomonas containing gelatin pellets were exposed to 0.6 parts/million of ozone for four weeks and the influence of this exposure measured. According to the preliminary data, exposure to ozone results in enhanced levels of infecting bacteria. Moreover, in addition to infection with the intratracheally induced Pseudomonas organisms, other bacteria, presumably from the rodents own flora, also proliferate within the lung. Pathological sections from the ozone-treated and control rats are presently being studied. If enhanced tissue damage in the chronically-infected rodents is observed, then this will be the first evidence of an adverse influence of ozone in an animal model reflecting a diseased state which is common in humans.

Mathematical models have been developed for aerosol deposition in the human respiratory tract. These models have shown accurate predictions of head, tracheobronchial and alveolar deposition after extensive comparisons with experimental data. They cover a wide range of particle characteristics and breathing patterns so that a concentration-dosage relationship can readily be established under various exposure conditions. The intersubject variability of deposition is important in the consideration of setting exposure standards. This variability has been studied using a probabilistic deposition model. It was shown that difference in airway dimensions is the single most important factor causing deposition variations, and it is possible to use two random scaling constants, one for the tracheobronchial region and the other for the alveolar region to account for this difference. Under this framework, the standard deviation of deposition for a population of healthy adults has been determined for various particle sizes at the normal breathing condition. The removal of the deposited particles in the tracheobronchial tree by mucociliary transport has also been studied with the use of an escalator model. This is an important mechanism for defending the lung. The mucociliary transport rates at various levels of airways are determined for the first time from particle retention curves. This method can be extended to study the effect of external agents on mucociliary clearance, and has the potential for use as a tool for airway size determination.

The practical value of a new microbiochemical assay procedure in research on vitamin E has been demonstrated. This technique was to freeze-dry cryostat sections of lung or micro-dissected parts thereof as source material for analyses by combined gas chromatography-mass spectrometry (GC-MS). As little as 3 ug of dried tissue are required for the analyses. Samples are weighed on a microbalance, placed in a small vial, an internal standard added, and they are then extracted and derivitized in one step with trimethylsilyl ether for

analysis by GC-MS. This new technique was used to determine the amount of vitamin E in the lungs of rats on formulated diets (i.e., 0, 200, or 3000 mg of vitamin E/kg of food) and age-related models (i.e., 10-day-old nursing pups, 90-day-old young adults, and 2-year-old aged animals) both before and after exposure to the oxidant gases NO_2 or O_3. Structural studies with the light and electron microscopes were conducted on lung tissue from the same animals as those used for vitamin E determinations. The data obtained from these studies have shown that the age-related animals have approximately the same amount of vitamin E in their tissue; however, the structural response in terms of cellular injury and repair are very different. The nursing pups are very resistant whereas the young adults are sensitive to cellular injury from oxidant gases. Aged animals are hypersensitive. Rats on vitamin E diets had very different levels of the vitamin in their lung tissues; however, their cellular response to injury and repair was indistinguishable. Since the theoretical role of vitamin E as an antioxidant in oxidant-exposed lungs is controversial, the significance of this research is that through direct analysis of the quantity of vitamin E in the tissue together with structural analysis on the same lung it has been shown that the response of lung cells to injury and repair is independent of the amount of vitamin E in the tissue.

Exposure of a transformed Syrian hamster embryo cell line to oxygen tensions above normal (50-95%) is cytotoxic. D_{37} was found to be an exposure to 79% oxygen for 48 hours. There is a 10-fold increase in mutation frequency at the hypoxanthine-guanine phosphoribosyl transferase locus upon exposure to 95% oxygen for 24 hours. The risk is substantially increased (D_{37} = 39% oxygen, 48 hrs.) when the cellular oxygen defense system is compromised by treatment with diethyldithiocarbamate (3mM, 1.5 hrs.) which reduced superoxide dismutase activity to 20% of the normal level. DDC is also cytotoxic to cells in 1 or 5% oxygen and in air. Cellular catalase activity was raised by incubation with loaded liposomes. This enhancement prevented hydrogen peroxide-induced cytotoxicity and strand scission. These experiments represent the first step in determining whether injury to cells is dose dependent as oxygen tension increases. Furthermore, the data demonstrate that the cellular oxygen defense system can be manipulated. Thus, studies can be conducted in low oxygen tension. The relationship between the oxygen defense system and the unavoidable risk due to exposure to oxygen can be carefully examined.

In a study on the health effects of Mount St. Helens volcano, rats are being exposed to 5 mg/m^3 or 50 mg/m^3 (respirable aerosol concentrations) volcanic ash, or to 50 mg/m^3 quartz, 6 hrs./day, 5 days/week, for up to 24 months. Subgroups of rats are killed at 4-month intervals to determine lesion development as a function of dose and cumulative exposure. After 4 months of exposure, the quartz-exposed rats had moderate diffuse alveolar proteinosis, patchy interstitial reaction that included prominently enlarged alveolar type II cells, increased numbers of alveolar macrophages and neutrophils, and pronounced histiocytic peribronchitis. The mediastinal lymph nodes had pronounced histiocytic lymphadenitis. The rats exposed to 50 mg/m^3 volcanic ash had significantly less pronounced lesions. The rats exposed to 5 mg/m^3 volcanic ash had only minimal changes, composed primarily of aggregates of dust-laden alveolar macrophages. The lungs of sham-exposed rats were normal. Mean lung weights of the groups, reflecting the histologic changes, increased in the following order: sham-exposed controls, 5 mg/m^3 ash group, 50 mg/m^3 ash group, and quartz-exposed positive controls. Other rats received one 60-min. nose only exposure to 135 mg/m^3 neutron-activated volcanic ash to determine pulmonary

deposition and clearance. Serial sacrifices, ranging from 15 min to 120 days post exposure, and γ-ray analysis of the lungs for radionuclide tracers indicate an initial alveolar deposition of 6% of the inhaled ash. After 5 days, 80% of this quantity was still in the lungs.

MUTAGENESIS

The mutagenic activities of cyclopenteno [cd] pyrene (CPEP), cyclopentano [cd] pyrene (CPAP) and seven derivatives oxygenated at the 3 or 4 positions of the pentano ring have been measured in diploid human lymphoblasts and Salmonella typhimurium. The direct-acting mutagenic derivative CPAP-3,4-oxide was significantly mutagenic to both S. typhimurium using resistance to 8-azaguanine as a genetic marker and to diploid human lymphoblasts employing resistance to trifluorothymidine as a marker in the concentration range of 0.4-0.7 uM. Incubation of CPAP-3,4-oxide in the presence of Aroclor 1254-induced rat liver PMS significantly reduced its toxic and mutagenic effect upon S. typhimurium and human lymphoblasts. However, when CPAP-3,4 oxide was incubated with purified rat liver microsomes in the bacterial mutation assay, there was no significant loss in toxicity or mutagenicity. CPEP and its other mutagenic derivatives all required metabolic activation to be detectable as mutagens to both S. typhimurium and human lymphoblasts. A striking synergistic effect occurs in the groups in which the two compounds were administered together, as can be observed from the latency period of tumor appearance, tumor incidence and the number of tumors. Since CPEP is abundant in auto exhaust and in urban ambient air, the carcinogenicity of these samples could be largely accounted for by CPEP, BP and their synergistic effect.

It was observed that when populations of single Chinese hamster ovary (CHO) cells were exposed to the mutagen ethyl methane sulfonate (EMS), allowed to grow into colonies and stained for glucose-6-phosphate dehydrogenase (G^6PD) activity, two types of unstained colonies were observed at a frequency of about one per thousand stained colonies. These negative-staining colonies consisted of (1) colonies uniformly deficient in staining activity (pure); and (2) colonies containing both stained and unstained sectors (mosaic) in various relative sizes and patterns. It is concluded that the simplest explanation for this phenomenon is that EMS induces a mutational change in one of the two DNA strands and DNA replication then produces normal and mutant double-stranded DNAs which segregate into wild-type and G^6PD-deficient cell types, producing a half-sectored colony. It appears that this is the first demonstration in mammalian cells that a significant proportion (27%) of mutations are fixed after the first cell division. Mutagen induction of mosaic colonies in a somatic mammalian cell line is a new finding which allows one the unique opportunity to study the early events of mutagenesis in mammalian cells. Prior to the sectoring phenomenon, the only mutagenic parameter of a chemical or physical agent that could be measured in mammalian cells was its effect on mutation rate. For the first time, one can monitor an agent's influence on a number of mutagenic phenomenon in addition to mutation rate; the ratio of pure mutant: sectored colonies, the distribution of colony sector sizes, the timing of the mutation fixation event, etc.

Both EMS and MNNG induced reversion of cycl-131 and cycl-115, two alleles which revert specifically by a GC to AT transition, the predominant change induced by both EMS and MNNG, were examined in stationary and exponentially growing cells, treated either in buffer or growth media. No significant difference in EMS

induced reversion of either cyc1 allele was observed in stationary vs. exponential RAD+ (wild type) strains treated in buffer. Similar results were obtained with MNNG as the mutagen, except that treatment of exponential cells with MNNG in media resulted in somewhat higher mutation frequencies than observed for exponential cells treated in buffer. In two repair-defective mutants examined, rad6 and rad52, very little, if any, induced reversion was detected with either EMS or MNNG, in exponential or stationary cells, suggesting that misreplication may play very little role in alkylation mutagenesis in yeast. No evidence for an adaptive response was found.

A method for the isolation of gamma ray sensitive Chinese hamster ovary cell mutants has been developed during the past year. This approach uses nylon cloth replica plating and photography with dark field-illumination to directly monitor colonies for growth after gamma irradiation. Using this method, two gamma ray sensitive cells were isolated. For one of these cells, the curve of the percentage of log-phase cells surviving increasing doses of gamma rays displayed two slopes: an initial steep slope and then a flattening of the curve at about 10% survival. Subsequently, it was found that the reason for this result is that this cell is sensitive to gamma irradiation in part of the cell cycle. This cell is sensitive in G-1, early S phase and late G-2 periods, while in the resistant phase (late S phase) its survival approaches that of the parental cells. The D_{37} of this cell in the sensitive G-1 period is approximately 30 rads which is about one-tenth the D_{37} of the parental cell (300 rads). This cell is the first gamma ray sensitive mammalian cell mutant which has been isolated directly on the basis of its sensitivity to ionizing irradiation. Also, it appears that this mutant is the only known cell-cycle dependent gamma ray sensitive mammalian cell obtained from any source. If this cell is defective in the repair of single strand DNA breaks during the sensitive cell cycle periods, it would immediately suggest the existence of at least two separate pathways for the repair of gamma ray-induced damage in mammalian cells.

Mutational effects of two transposable elements in Drosophilia melanogaster are being studied. One of these is the P-factor, which causes the syndrome of hybrid dysgenesis. The other is the L-factor, known to be responsible for the induction of lethal mutations and chromosome breaks in an "unstable" X chromosome. The work with the P-factor dealt primarily with an anomolous Drosophilia strain known to harbor several copies of the P-factor, but which does not exhibit all the properties of normal P-strains. P-induced mutations occurring on the X chromosomes of descendants of this strain are restricted to very few sites. The remarkable specificity of the mutation process suggests that these sites harbor P-factors that are incapable of transposing to another location. The L-factor is an element that resides near the cut locus on the X chromosome of one Drosophilia strain, and possibly at a few other locations. It causes X-linked lethal mutations and chromosome breaks, and also seems to catalyze the formation of compound X chromosomes through the splicing of segments near the centromere. This factor seems to move from the unstable X chromosome to a homolog independently of ordinary recombination, as judged by the homolog's acquisition of the properties of the unstable chromosome.

Cell killing and mutation induction to 6-thioguanine resistance, ouabain resistance and diphtheria toxin resistance by MNNG were determined in asynchronous and synchronous CHO cells. In asynchronous cells the survival curve had two components, an initial steep drop and then a flatter part. The dose response of induced mutants, on the other hand, was linear. Therefore, cell killing and

mutation induction by MNNG seem to be independent. When synchronous populations with cells 1 hr. apart in the cycle were treated with MNNG, cell killing varied depending on the time of treatment. Cells in G_1 were the most sensitive, and cells in late S, the most resistant. The induction of mutants to the three genetic markers was independent of cell cycle time. Therefore, the flat response which had been observed with ENU seems to be typical for alkylating agents in this cell line and might be related to the inability of these cells to repair the potentially mutagenic O^6 alkylaguanine formed by alkylating agents.

In a study of the use of genetic assays in Drosophilia, it was shown that the X-linked recessive lethal assay on germ cells, in combination with a somatic mutation assay, provides an efficient means of screening a large number of chemicals in Drosophilia. Experiments with some aromatic amines and polycyclic hydrocarbons, two classes of carcinogens which give only marginal effects when assayed on germ line tests, support the view that somatic assay systems are valuable as a complement to the recessive lethal test. Ten carcinogens were assayed for the induction of somatic mutation and recombination in the w/w^{co} system, and all ten gave a positive response.

There is a general direct correlation between chromosome-breaking efficiency, the occurrence of delayed genetic damage and N-alkylation in DNA, and a general inverse association between this parameter and the ability of AAs to induce point mutations. Ability to cause chromosomal aberrations and delayed mutations decreased in the sequence: MMS > DMS = MNU > DMN > EMS = DES > iPMS ENNG = DEN = ENU. Conversely, those AAs acting more extensively at the oxygen atoms in DNA, namely ENNG, DEN and ENU, while being less active with regard to the production of breaks, are more potent in inducing point mutations. Much attention has also been drawn to the properties of xenobiotica-metabolizing enzymes in Drosophilia. The presence of several types of cytochrome P-450 and considerable aryl hydrocarbon hydroxylase activity in microsomal preparations of Drosophilia were demonstrated. Furthermore, benzo(a)pyrene hydroxylation, paranitroanisole demethylation and epoxide hydratase catalyzed reactions were also used to characterize xenobiotica-metabolizing enzymes in different Drosophilia strains and developmental stages (larvae vs. adult flies).

The Chinese hamster ovary cell line (CHO) is being employed to develop a cytogenic assay for environmental chemicals. A protocol of treating the cultures with agents for five hours and harvesting the cells for cytogenetic analyses could satisfactorily provide answers to these two questions. (1) Does this chemical cause chromosome breaks? and (2) If so, how much? Experiments were also conducted with laboratory mice to analyze drug effects on male gametogenesis. With one sublethal intraperitoneal injection of adriamycin, testes became atrophied with no recovery. At lower doses, a recovery was noted after a period of temporary cessation of spermatogenesis, but chromosome aberrations were still noted in the recovering spermatocytes. In the course of this study, human lymphocyte cultures were occasionally used as supplementary material. It was found that lymphocytes of different donors responded to drug treatments with different frequencies of chromosome breakage. This phenomenon was later expanded to provide evidence for developing a hypothesis that in the human population, genetic instability is not an all-or-none phenomenon.

Through careful measurement of sperm phenotypes environmental damage to germ cells can be detected. From the human point of view the study of germ cells in

the testis is not practical, while human sperm can readily be obtained and evaluated. Mice were used in the present model study, however, methodology worked out in the mouse sperm is applicable to the study of human sperm. A simple and highly reproducible assay for the sperm enzyme acrosin, an enzyme necessary for fertilization, in single mouse spermatozoa was developed. The same assay also works with human, monkey, dog, rabbit and rat sperm. With the newly developed acrosin assay damaging effects of ethylnitrosourea, cyclophosphamide and mitomycin C in spermatogonial and pre-leptotene cells in mice were detected. The effects of these compounds were also detected by sperm motility, sperm count and sperm morphology in the same groups of mice. The newly developed enzyme assays will be useful not only for detecting developmental and mutagenic damage to mammalian germ cells, but may also help to detect environmental agents which induce reproductive dysfunction.

Animal studies are being conducted to evaluate the carcinogenicity of combined exposures to HCl and HCHO either premixed or non-premixed, HCHO alone, and HCl alone; and analytical observations to evaluate the formation of BCME from HCl and HCHO under dynamic conditions. Preliminary results indicate the combined exposure to HCl (11 ppm) and HCHO (15 ppm) are decisively positive for the induction of squamous cell carcinomas of the nasal cavity. Much of the carcinogenic response is from HCHO since HCl exposure did not produce any tumors when given alone. These results confirm those in the CIIT study; that HCHO is a potent respiratory carcinogen. The malignant tumor response from HCHO alone (40%) and from HCHO plus HCl premixed at high concentrations (48%) are nearly similar. The combination of HCl and HCHO when not premixed, yielded a much lower response (29%). This suggests that the level of 15 ppm may be saturating the carcinogenic responsiveness of the nasal mucosa. At this concentration, the added irritation of HCl might simply suppress the tumorigenic response. The induction of only one esthesioneuroepithelioma suggests that the level of BCME in the premixed combination is not a factor in the tumorigenesis.

The mechanisms of silica toxicity to mouse macrophage $P388D_1$ cells in vitro is being investigated. Two metabolic changes have been evaluated in relationship to silica toxicity: ATP depletion and lysosomal integrity. ATP depletion occurs in $P388D_1$ cells exposed to silica, but not to nontoxic particles, in the presence of extracellular calcium and accompanies cell death. ATP depletion can also be produced by a combination of metabolic inhibitors; however, there is no loss of viability although there is intracellular lysosomal rupture. While ATP depletion in itself can cause intracellular lysosomal rupture, phagocytosis of silica particles in the absence of extracellular calcium is also associated with release of lysosomal contents without depletion of ATP or loss of viability. These observations dissociate ATP depletion and intracellular lysosomal rupture from the mechanism of killing of $P338D_1$ cells by silica particles. Silica particles are hypothesized to interact directly with both the plasma and lysosomal membranes. Interaction with lysosomal membranes results in intracellular release of lysosomal contents, but not necessarily in irreversible cell injury. Interaction with the plasma membrane leads to calcium influx which is followed by cell death and ATP depletion.

METALS

Lead poisoning appears to damage the developing central nervous system primarily through its effects on the development of the microvasculature. There are

direct toxic effects on developing neurons but the major alterations are secondary to vascular injury. Ultrastructural studies, using horseradish peroxidase as a tracer, have shown permeability changes in the neonatal rat cerebellar microcirculation beginning 24 to 48 hours after the administration of lead acetate via an esophageal catheter. The damage to the "blood-brain barrier" becomes progressively more severe with continued lead administration. The morphologic alterations include disruption of endothelial cell junctions, formation of microaneurysms and reduplication of endothelial cells.

Operationally defined hyperactive children with lead levels from 25-55 ug/dl were treated with placebo, methylphenidate or penicillamine in a double blind treatment protocol. Children on penicillamine improved significantly when compared to the placebo group and did not differ from the methylphenidate group (the drug of choice) at the end of treatment. The evaluative measures included those from the children's teachers, parents and doctors. Blood lead as a measure was evaluated for stability over a three month period. Intercorrelation over that period (taken at monthly intervals) were all approximately 0.8 indicating that blood lead over that period of time is a relatively stable measure. Mentally retarded children were screened for increased blood lead levels. Data indicated that (a) those children who had no probable cause for their condition had significantly higher blood lead levels than did a normal control group; (b) mentally retarded children with a good reason for retardation that was not lead related had lead levels no different than a normal population (c) a positive Pearson correlation of .28 was found between I.Q. and blood lead levels in the above group.

The effects of lead on molecular structures and functions in the body are poorly understood. A longstanding cytological observation is the appearance of intranuclear inclusion bodies in the proximal kidney tubules of lead-intoxicated animals. In order to understand the significance of these inclusion bodies, they have been isolated and their constituent proteins characterized. A predominant protein appears to be uniquely located in the inclusion bodies. The identification of a protein unique to the inclusion bodies suggests that inclusion body formation is a programmed event induced by lead and is a protective response to lead exposure. This possibility is being tested by examining the induction and metabolism of the unique protein.

A model system in which to study the effects of chronic low level lead exposure on the physiology of individual nerve cells is under development. The pond snail Lymnaea stagnalis has been chosen for this research because of its large, well-characterized, individually identifiable neurons. It was found that chronic exposure to low levels of lead causes behavioral deficits in feeding in these animals. Neurophysiological studies of the brains of animals sacrificed during the time of behavioral deficit have found that some, but not all, parameters of nerve cell electrophysiology have been altered by lead. In general, all nerve cells from lead-exposed animals, when compared to controls, show an increased resting potential (-53.1 $\pm$ 0.7 mV vs. -50.0 $\pm$ 0.7 mV; means $\pm$ std. error); slowed rate of recovery of the undershoot of the spike (22 $\pm$ 2% of the undershoot recovered in 100 msec vs. 33 $\pm$ 2%); reduced spontaneous activity (0.16 $\pm$ 0.04 Hz vs. 0.30 $\pm$ 0.04 Hz); and reduced input resistance (43 $\pm$ 2 Megohm vs. 50 $\pm$ 2 Megohm). In addition, different types of identifiable neurons also show differential lead effects, especially on intrinsic excitability as measured by spike frequency elicited by intracellular current injection. Three different

neuron types, termed the B, F, and LP1 cells, show markedly depressed excitability when lead-exposed, whereas the excitability of the RPeD1 and VV1/2 neurons is unchanged. These results suggest that chronic lead alters specific characteristics of nerve cell electrophysiology, which would in turn alter neuron functioning and behavior. In addition, different types of neurons, with different membrane characteristics, are differentially affected by lead. Thus, the effects of lead on "the nervous system" will depend upon the characteristics of the particular neuron under study. These studies are the first both to demonstrate lead effects on many physiological parameters and to show clearly that electrophysiological parameters of different types of neurons are affected differently. In addition, this model system has the advantage of easily studying the ionic mechanisms by which lead exerts its effects. Future experiments will perform similar analyses on synaptic transmission between identified neurons.

Lead (3 uM) perfusion of the myocardium resulted in a pronounced increase in coronary vascular resistance which suggests that lead hypertension may be induced in vivo by a direct action of lead on vascular smooth muscle. The action of lead on myocardial metabolism was characterized by a pronounced decrease in tissue creatine phosphate levels with a corresponding accumulation of inorganic orthophosphate. In contrast to the effects observed for cadmium, lead induced only a modest reduction in myocardial ATP levels. The observed metabolic and physiologic actions of lead suggest a fundamental mechanistic difference between cadmium and lead regarding the cellular actions of these heavy metals in the heart. The effects of lead were not consistently antagonized by increased extracellular calcium concentrations suggesting that a more complex cellular mechanism involving more than a direct competition with calcium is involved.

Two indices of adrenergic nervous development, norepinephrine uptake as an index of presynaptic component development, and beta-adrenoceptor population and binding affinity as an index of postsynaptic component development, have been examined to determine the mechanism by which neonatal lead exposure caused cardiotoxicity in rats. Neuronal norepinephrine uptake capacity develops at a similar rate in lead-exposed and control pups, but there is some indication that the vesicular uptake process may be altered. The development of beta-adrenoceptor binding capacity and number is not altered by lead exposure. Recent preliminary experiments, however, indicate that the proportions of beta-1 and beta-2 receptors in the heart may be affected by lead.

The administration of picrotoxin to untreated, urethan-anesthetized rats causes a slowly developing increase in blood pressure, and a transient decrease in heart rate. Pb-exposed animals respond to picrotoxin with a similar blood pressure effect, but a more pronounced, longer lasting bradycardia. The possibility that this observation may reflect Pb action on brainstem GABAergic receptors in parasympathetic pathways is under study.

In experiments designed to generate a simpler model for the study of lead cardiotoxicity than the chronic model currently in use, large differences have been observed in the concentrations of lead required in vitro and in vivo to cause toxicity. Administration of 10^{-6}M Pb to isolated rat hearts causes significant decreases in cardiac rate and contractility, while intravenous infusion of Pb into anesthetized rats in amounts to produce blood Pb concentrations as high as 10^{-4}M has minimal effects, causing only a small decrease in

heart rate. The uptake of Pb into heart tissue in these experiments is greater *in vitro* than *in vivo*. Although it is commonly thought that Pb in blood is approximately 90% bound to erythrocytes, plasma Pb values in these experiments were also quite high, suggesting that some form of plasma binding might be exerting a protective effect *in vivo*.

Bone is the major reservoir of the body burden of lead in humans, yet the biological significance of bone Pb is largely unknown. To understand further the complex interactions between skeletal lead, lead chelating agents and bone cell metabolism, lead transport in two *in vitro* systems: bone organ culture and isolated bone cell populations (osteoclast-like (OC) cells and osteoblast-like (OB) cells) are being studied. During the past year, a rapidly exchangeable skeletal lead compartment that is regulated by the same ions and hormones that control bone cell metabolism under physiological conditions have been characterized and defined. The results of studies showed that ^{210}Pb uptake by OC and OB cells was remarkably rapid in a linear manner: maximum uptake occurred by 2.5 to 3.0 hours in both types of bone cell populations. Double labelling experiments with ^{210}Pb and ^{45}Ca indicated that the quantity and time course of Pb and Ca uptake were remarkably similar in OC and OB cells. It was also found that parathyroid hormone (PTH) significantly increases the uptake of ^{210}Pb in a manner similar to the time-dependent increase in Ca uptake by OC cells; and this effect of PTH on ^{210}Pb uptake was responsive to graded concentrations of the hormone. From these data, it appears that Pb and Ca uptake are coupled. It can be anticipated that a significant fraction of bone lead may be readily mobile *in vivo*, thereby serving as a large endogenous source for critical soft tissue sites (brain, blood, liver...) under physiological conditions.

To resolve the long-standing controversy concerning the quantity of accumulated Pb that produces clinical and subclinical toxicity, a practical, sensitive, non-invasive technique for measuring bone Pb directly is needed for population screening. Successful X-ray fluorescence (XRF) prototype instruments have been developed. A high correlation (r=.92) between *in situ* XRF measurements and those obtained by atomic absorption spectroscopy (AA) from carefully removed bone samples from the same area encompassed by XRF determinations has been found. Similar comparison of AA vs. XRF measurements *in situ* reveals a correlation coefficient of 0.97 - at bone lead concentrations at or slightly below 10 ug/gm. Clinical testing of such instrumentation to measure bone Pb stores *in vivo* will now begin. By correlating highly sensitive biochemical effects of lead on vitamin D and cortisol metabolism with precise measures of bone lead stores by XRF, it will be determined if there is a threshold concentration for lead's toxic biochemical effects in children.

M. fasciularis females given 0, 50 and 90 ug/kg b.w. methylmercury (MeHg) orally daily throughout gestation produced three significant (previously unrecognized in human or subhuman primates) findings in the offspring: (1) the 90 ug group had a reduced conception rate and increased fetal mortality rate with two first trimester abortions; (2) infants born to mothers exposed to MeHg were smaller than the nonexposed, and the post-natal development of object concept (Piaget) was delayed in all MeHg exposed. (3) Consistent with this is the neuropathologic demonstration of focal neuron necrosis in the brains of infants at these dose levels throughout the cortex as a result of congenital MeHg exposure. These findings were observed even though at the 50 & 90 ug dose levels gross behavioral observations were normal. No "overt clinical" toxic symptoms or signs were observed in the mothers or offspring at these dose levels. This is

the first demonstration or harbingers of injury at dose levels midway between "background" and clinical toxic levels. It was shown that MeHg impedes mitosis and that assembly of microtubules, the principle component of the mitotic spindle, is impeded by MeHg.

Selected heavy metals, such as mercury and cadmium have been found to adversely effect transport of amino acids across the human placenta. The placenta as a target organ is being studied to determine the role of placental toxicity in chemical-induced fetal demise. Further, a nonneuronal cholinergic system has been discovered in the human placenta and preliminary evidence indicates it is functional in regulating fetal growth. Environmental toxicants which have been determined to perturb this system are known to produce adverse effects in the human fetus. The mechanism of certain environmental toxicant-induced teratogenic effects may be an alteration of the placental cholinergic system. A similar nonneuronal cholinergic system was identified in human sperm. Perturbation of this system by environmental toxicants was found to adversely affect reproduction.

The molecular basis of the effects of methyl mercury (MeHg) on cellular DNA and RNA synthesis is being studied. MeHg has previously been shown to inhibit DNA and RNA synthesis in intact HeLa cells. It was determined that in isolated nuclei MeHg inhibits DNA synthesis but stimulates RNA synthesis. Several experiments to eliminate possible artifactual explanations for this differential effect were carried out, such as, determining the effects of MeHg on the level of the triphosphate precursors in the reaction or on nucleic acid hydrolysis by nucleases. It was found that the stimulation by MeHg is specific for synthesis which is catalyzed by RNA polymerase II. In contrast, synthesis catalyzed by polymerases I and III is completely inhibited by MeHg. In order to determine whether this differential effect of MeHg on the RNA polymerase activities in isolated nuclei also occurs with the soluble enzymes, an extract of HeLa cells was prepared. In contrast to the situation in isolated nuclei, both alpha-amanitin sensitive and resistant synthesis in the extract were inhibited by MeHg. In addition, RNA polymerase II which was further purified by DEAE-sephadex chromatography was also found to be inhibited by MeHg. However, if the enzyme is pre-incubated with reaction mixture (containing the DNA template and the ribonucleoside triphosphates), subsequent synthesis is resistant to MeHg and is even somewhat stimulated by it. In contrast, pre-incubation had no effect on the inhibition of polymerases I and III by MeHg. The simplest explanation for these observations is that MeHg inhibits initiation of RNA chains by all three polymerases, as well as elongation by polymerases I and III. In contrast, elongation by polymerase II was not inhibited by MeHg and was even stimulated by it at some concentrations. Since only chain elongation occurs in isolated nuclei, this result explains the effects of MeHg on RNA synthesis in isolated nuclei and in whole cells.

A research effort was concentrated on (a) extending comparative studies of antidotes to organometallic compounds of mercury (such as CH_3HgCl and C_6H_5HgOAc) (b) the synthesis of new sulfhydryl containing ligands capable of removing mercury and methyl mercury from bound sites within cells and (c) development of analytical capabilities to determine various types of mercury in the organs of experimental animals. The studies on antidotes for CH_3HgCl led to the conclusion that dimercaptosuccinic acid was the most effective of the antidotes available, but that its efficacy was less than desirable. The damage caused by acute CH_3HgCl seems more difficult to reverse than that due to inorganic mercury

compounds. It was also found that a dimethyl sulfoxide solution of CH_3HgCl could be used to obtain a means of administering known amounts of the compound which were more reproducible than the aqueous solutions previously used. Since organomercury compounds can penetrate cell membranes, sulfur containing compounds which can also pentrate and then split to form -SH containing molecules which are more effective in reacting with the methyl mercury moiety have been synthesized, but the compounds of this sort prepared so far have only a modest antidotal activity.

The mechanisms of action of methylmercury on the nerve and muscle system is under study. The effects of methylmercury on ionic channels and receptors of neuroblastoma cells and on neuromuscular transmission in the rat are being investigated by identifying and characterizing various ionic channels and receptors in neuroblastoma cells. Internal perfusion and voltage clamp techniques were developed which permitted clear-cut separation of sodium, potassium and calcium currents. Three components of acetylcholine receptors and channels were identified and characterized using microelectrode and voltage clamp techniques and also using specific toxins as probes. Dopamine receptors and channels were also studied in detail. Furthermore, a patch voltage clamp technique was successfully developed to record single sodium channel activity, the technique which can be applied to other channels such as calcium, potassium and receptor associated channels. Experiments on the effects of methylmercury on the ionic channels and receptors of neuroblastoma cells have also been performed.

Experiments were designed to test the hypothesis that copper and/or zinc, endogenous components of chromatin, are associated with nucleoprotein fragments which participate in certain hormonal responses. Of special interest is the finding that endonuclease digestion of chromatin yields fragments enriched in "linker DNA" and these nucleoprotein particles are stable only when endogenous divalent metals are present. II. Metallothionein (MT) studies in which both mammalian (rat) and non-mammalian (chick) fetal and neonatal tissues were utilized demonstrated that elevated levels of endogenous MT-like proteins appeared as early as 15 days post-fertilization, reached a maximum plateau 2-6 days after birth and declined to adult levels 28 days later. This pattern has been observed in both rat and chick liver and in rat pancreas and spleen. By comparing MT developmental profiles in such a system, it is hoped that new insights into physiological function(s) of these copper and zinc binding proteins will emerge. The observation that the initial partitioning of cytosolic zinc with the MT fraction can be correlated in fetal rat liver with a cellular shift from proliferation to differentiation, merits special attention.

A determination was made as to whether antioxidants decrease the toxicity of four chemicals (sodium iodoacetamide, diethyl maleate, sodium vandate, and (copper), known to produce lipid peroxidation in isolated hepatocytes. In general, antioxidants decreased the lipid peroxidation caused by these chemicals but not their toxicity. This suggests that lipid peroxidation is not the cause of the injurous effects of these chemicals. Cd was shown to be rapidly removed from the blood but 12 hrs. after IV injection the concentration increased. The increase was mainly in the red blood cells. The Cd was evenly distributed between the RBC ghosts and the cytosol. The Cd in the cytosol does not appear to be bound to metallothionein. The effect of various chelators in decreasing the body burden of Cd was determined. Significant increases in survival and excretion and a decrease in tissue levels of Cd was produced by a number of

chelators but ethylenediaminetetraacetic acid was the most effective. The induction of metallothionein was investigated to determine if steroids could induce its synthesis and if its synthesis might be regulated by glutathione. Hydrocordisone and dexamethasone increased metallothionein concentration in the liver but this was a small increase (40-80%) in comparison to that seen after Cd exposure (2000%). It was also found that tissue levels of metallothionein and glutathione levels are not interrelated.

The effects of cadmium on selected aspects of the immune response are being studied in laboratory mice. Animals are fed cadmium chloride in their diet and then screened at intervals for their ability to clear injected aggregates of immunoglobulin G (Agg-IgG). These aggregates are commonly used as models of antigen-antibody complexes. The body normally clears antigen-antibody complexes daily as a result of constant immunological encounters with foreign materials. An earlier finding in this laboratory showed that there is a significantly slower clearance of Agg-IgG in mice fed cadmium as compared to age-matched mice not receiving additional cadmium in their diet. Furthermore, there is about a 50% reduction in the liver's ability to remove Agg-IgG from the blood in mice on the cadmium diet. This effect was specific since removal of aggregates of bovine serum albumin, of similar molecular weight, was not affected by the cadmium diet. Also, noted was a 20% prolongation in sleep time, compared to controls, in cadmium-fed mice which received sodium pentobarbital.

In one study the major accomplishment during the past year has been the synthesis of chelating agents of very low toxicity which are able to remove cadmium from aged deposits in the kidneys of experimental animals. Since kidney accumulation and damage is such a prominent aspect of human chronic cadmium intoxication, these results are very encouraging in opening up possibilities for the therapy of this condition, which is currently untreatable in any but a supportive manner. The compounds which were prepared and used for this purpose are a number of water soluble dithiocarbamates which have structures that insure that their complexes with cadmium are also specific with a certain minimum amount of water solubility. The results using mice with chronic cadmium intoxication, show that, depending on the compound used, up to 90% of the cadmium present in the kidneys can be removed by a regime of chelate administration lasting three weeks. The further advantage of these compounds is that they do not carry this mobilized cadmium to more sensitive areas, such as the brain. The ability of the various compounds to remove cadmium from other organs appears to be strongly dependent on the structure of the chelating agent.

It has been found that certain chelating agents are able to mobilize small amounts of aged cadmium deposits when administered at therapeutically feasible levels. The amounts of cadmium mobilized are quite modest but do come, in part at least, from the kidneys. Because renal damage and renal failure play such an important role in human cases of chronic cadmium intoxication, these results are important in showing the possibility of a long-term treatment to lower kidney levels of cadmium and possibly restore kidney function. Studies on antidotes for acute cadmium intoxication have also shown that DTPA (diethylenetriaminepentaacetic acid) is the most effective antidote (given as the Zn or Ca salt) for immediate administration. It has also been confirmed that diethydithiocarbamate is a more effective antidote when given two hours after the cadmium than when given one hour after. Indications are that the same is true of EDTA. One possible reason for this is that the procudure utilizes endogenous as well as exogenous detoxification processes more efficiently.

Acute cadmium toxicity is being studied in vitro in a cell culture exposure system. Alveolar macrophages are isolated from adult New Zealand white rabbits (5-6 lbs.) and exposed to $CdCl_2$ in a primary cell culture system. With the use of erythrosin B exclusion as the index of cell viability, the effects of several culture parameters were investigated. These studies were conducted initially in order to characterize Cd toxicity in this system in a general manner. The parameters that have been examined include: the concentration of Cd in the culture medium; the time of incubation (exposure); the temperature of incubation; the type of culture medium utilized; the age of the animals from which the cells were obtained; protection against Cd-mediated cell damage by other, nutrient metals; and the effect of culture medium sterility. In an eight-hour exposure period (25°C), an LD_{50} value for $CdCl_2$ was determined to be 1000 uM, when the cells were cultured in HEPES-buffered Hank's Balanced Salt Solution (HBSS). When the cells were cultured in a complete nutrient medium (Ham's F12), an LD_{50} value for $CdCl_2$ greater than 2000 uM was found. This difference was subsequently investigated and was attributed to Cd complexation by inorganic phosphate. Exposure of cells to Cd in specially prepared Ham's F12 medium (w/o phosphate) produced an LD_{50} value for $CdCl_2$ of 1100 uM, not significantly different from the value obtained with the HEPES-buffered HBSS. Macrophages from younger animals (1 - 2 lbs.) were found to be more resistant to $CdCl_2$ toxicity, whereas those isolated from older animals (9 - 10 lbs.) were found to be more sensitive. Zn and Se partially protected the cells from Cd when present at stoichiometric amounts to Cd in the culture medium, whereas Fe^{2+}, Fe^{3+} and Cu did not. Both Fe^{2+} and Fe^{3+} were highly toxic to these cells. The addition of Staphylococcus aureus 502A to the culture medium significantly potentiated Cd toxicity.

Low level cadmium (1.0, 0.1 ppm) and/or lead (1.0, 0.1 ppm) administered via the drinking water to laboratory rats did not induce any overt or acute toxicologic manifestations after three months. However, the average conscious blood pressure values for each heavy metal exposure group were elevated significantly. The most pronounced hypertensive response was detected in the group given 1 ppm cadmium in the drinking water. Subsequent six month in vivo physiologic and tissue biochemical analyses performed on these same groups of rats were in progress. Parallel in vitro intact isolated perfused rat heart studies were conducted to examine the mechanistic bases for the actions of cadmium and lead within cardiovascular tissues. Analyses of the physiologic actions of cadmium demonstrated a concentration-dependent reduction in the physiologically active calcium concentration within the heart. Noteworthy among the physiologic actions of cadmium in the intact heart was the cadmium-induced increase in coronary vascular resistance which suggests a possible direct pressor mechanism of cadmium on arterial smooth muscle. The cellular actions of cadmium indicate that this heavy metal is a competative inhibitor of intracellular calcium-dependent processes and that the relative binding affinity of cadmium ions for cellular calcium-binding sites is 2000 times higher than the affinity of calcium for these same sites. In conjunction with these physiologic effects, cadmium caused a partial uncoupling of oxidative phosphorylation in the intact isolated perfused rat heart with a concomitant increase in tissue glycolytic activity. This action of cadmium was directly calcium-dependent. The detection of these metabolic and physiologic alterations coupled with their demonstrated calcium dependence provides experimental evidence suggesting that cadmium may act to depress myocardial function and metabolism in vivo through a calcium-mediated mechanism which involves intracellular sites rather than just superficial membrane actions. In contrast to the demonstrated cadmium-calcium antagonism,

the lead-induced modulation of cardiac physiologic and cellular processes was only transiently influenced by increased extracellular calcium levels and was followed by a latent synergistic interaction at higher extracellular calcium concentrations.

Carcinogenesis tests of 16 relatively insoluble nickel compounds (nickel sulfides, selenides, arsenides, oxide, antimonide, telluride, and titanate) have been completed or are underway following administration to groups of male Fischer rats by intramuscular (im) injection (14 mg Ni/rat) and by intrarenal (ir) injection (7 mg Ni/rat). Althrough the final outcome of these tests will not be available until 1983, observations to date demonstrate that Ni dust, Ni_3S_2, crystalline NiS, NiS_2, Ni_3Se_2, NiTe, NiSb, NiAsS, $Ni_{11}As_8$, Ni_5As_2, Ni_4FeS_4 and NiO are carcinogenic by the im route, and that Ni_3S_2, NiSe, NiAsS, $Ni_{11}As_8$, and Ni_4FeS_4 are carcinogenic by the ir route. Based upon preliminary results of the carcinogenesis tests, the carcinogenic activities of the nickel $Ni_{11}As_8$, and Ni_4FeS_4 are carcinogenic by the ir route. Based upon preliminary results of the carcinogenesis tests, the carcinogenic activities of the nickel compounds are not correlated with their dissolution rates or their susceptibilities to phagocytosis by rat macrophages; on the other hand, the carcinogenic activities of the nickel compounds are significantly correlated ($P < 0.0001$) with their potencies for induction of erythropoietin-mediated erythrocytosis after ir injection. Development of erythrocytosis in rats following ir administration of Ni_3S_2 requires the continued presence of the injected kidney, suggesting that enhanced erythropoietin production is a local response to the presence of Ni_3S_2 particles in the renal parenchyma. A new, sensitive method has been developed for measurement of microsomal heme oxygenase activity, based upon gas chromatography of carbon monoxide produced in vitro by enzymic degradation of heme.

Molybdenum centers with the sterically constraining hydrotris (3,5-dimethypyrazolyl) borate ligand were synthesized. Monomeric molybdenum(V) complexes of this ligand are unusually stable and provide a convenient series of benchmark molecules for systematic study of the relationship of the electron paramagnetic resonance (EPR) spectra of molybdenum(V) complexes with chemical structure. Such data will provide a basis for interpreting the wealth of EPR available data from molybdenum enzymes. Reaction of $Mo(CO)_3\{L\}$- (where L is the ligand noted earlier) with sulfur produced the novel compound $[Mo(CO)_2\{L\}]_2S$ which contains a linear Mo-S-Mo entity and a very short Mo-S distance (2.20 A). This distance is similar to the Mo-S distance observed in the molybdenum enzyme xanthine oxidase by extended x-ray absorption fine structure (EXAFS). Attempts to synthesize complexes with molybdenum sulfur bonds led to the unexpected discovery of catalytic oxidation of thiols to disulfides and the catalytic oxidation of sulfite. The latter reaction may be important for understanding the mechanism of the enzyme sulfite oxidase.

The effects of heavy metals on tissue metal content, biotransformation enzyme activities and peripheral motor nerve conduction velocity were studied. Manganese exposed rats showed an adaptation in one week despite continuous exposure via drinking water. Also, both the polysubstrate monooxygenases and epoxide hydrolase activities were enhanced in one week in correlation with the increase in the manganese concentrations in hepatic, intestinal, blood, cerebral and renal tissues. When the interaction of simultaneous zinc administration was

studied in lead exposed rats, zinc was not able to prevent lead-induced decrease in the peripheral motor nerve conduction velocity.

Exposure of rabbits to systemic aluminum during gestation (5 sc injections weekly for four weeks of 0, 25, 100 or 400 umole Al/kg/injection) resulted in 58% mortality of the offspring at birth or by 48 hours after birth in those exposed to 400 umole Al/kg. No mortality was seen in offspring exposed to lower aluminum levels. No large effects were observed in surviving offspring growth rates. Offspring of does receiving 25 umole Al/kg demonstrated facilitation of acquisition of a classical conditioned response (nictitating membrane extension) when conditioned at six and ten weeks of age. There was a suggestion of attenuation of acquisition of learning in offspring of does receiving 400 umole Al/kg when conditioned at ten weeks of age. Milk aluminum levels during lactation were elevated in those does exposed to aluminum during gestation, dropping off over time. Exposure of rabbit does to the above schedule of aluminum injections during lactation resulted in a doubling of milk aluminum levels at the highest aluminum exposure level, compared to controls. Offspring of does receiving 400 umole Al/kg demonstrated lower body weight gain than controls (15 to 30%) throughout the 12 weeks of the study. Milk consumption by these offspring averaged 75% compared to controls, based on milk weight consumption determined every five days of lactation. Body weight of does receiving 400 umole Al/kg during gestation or lactation decreased 10% compared to their pre-exposure body weights whereas controls gained 5% body weight. This weight loss is comparable to that seen in non-pregnant non-lactating does, suggesting that pregnancy and lactation does not protect against aluminum-induced body weight loss. These results suggest that systemic exposure to excessive amounts of aluminum during gestation or lactation can have adverse effects, especially on perinatal mortality and growth, although profound behavioral deficits have not been seen under these exposure conditions.

PHYSICAL FACTORS

NOISE

Additional anatomophysiological studies in monkeys exposed to repetitive, moderately intense stimuli (daily doses) of 3-min, 100-dB pure tones) demonstrated permanent functional and structural damage to cochlear nucleus and cochlear sensory cells, respectively. Such permanent changes to the auditory apparatus indicate that so-called "safe" sounds may be harmful if regularly repeated over a lengthy period of time. Careful microscopic examination of subtly damaged hair cells will provide methods for predicting potentially damaging sounds in the environment.

ELECTROMAGNETIC FIELDS

The primary objective of a study underway is to answer the question: "Is the cell membrane altered by 60 Hz electromagnetic fields?" A novel technique for characterizing the cell surface which is based both on cell surface charge and cell surface hydophobicity was developed. The technique makes use of the fact that when the water-soluble polymers dextran and poly-(ethylene glycol) are dissolved in sufficiently high concentrations, two aqueous phases are formed. One is dextran rich, the other is rich in PEG. When cells are added to such a

two-phase system (which contains salts to make it isotonic) they partition between the two phases and the interface in a manner that is strongly dependent on cell surface properties. Using this technique a cell surface effect due to weak 60 Hz electromagnetic fields was shown. It appears that this is the first direct observation of a cell surface effect due to 60 Hz fields. Additional studies indicate that countercurrent distributions can be used to "fingerprint" cell populations exposed to fields in a manner that should prove useful in unraveling the mechanism by which electromagnetic fields interact with living cells.

MICROWAVE

Experiments indicate that in mice exposed for 10 minutes near field microwave irradiation (2.54 GHz, 10 mW/cm^2), blood brain permeability is increased. The irradiation causes the mice to lose the righting reflex more quickly to phenobarbital than sham-irradiated mice. The effect is associated with a more rapid uptake of phenobarbital into the brain. Since a similar rise in rectal temperature of 1-2° C produces parallel results, the mechanism of the effect is largely thermogenic. In a different experiment, the antagonistic effect of methyl atropine was tested against the hypothermia induced by pilocarpine and the tremors produced by oxotremorine. These central nervous system effects were not antagonized by methyl atropine in control mice but were blocked by methyl atropine in the microwave irradiated mice. Since normally methyl atropine does not gain access to the central nervous system, the microwave irradiation had increased the access of methyl atropine to the brain (increased blood brain barrier permeability).

Given sensory guidance in the form of visible light that accompanies behaviorally contingent bouts of irradiation, mice and rats eventually learn to escape from extremely intense irradiation. Once such learning occurs, the visiblelight cue can be omitted and the animal will not only escape from but will repeatedly re-enter and leave the field, achieving, thereby, a finely maintained level of thermoregulation. The average rate of self-selected energy dosing by a mouse at an environmental temperature of 25 °C exceeds 40mW/g, a level that is 500 percent highter than the animal's resting metabolic rate and that would be lethal within 15 minutes if irradiation were continuously applied. Since such control eliminates the psychogenic stress associated with investigator-controlled periods of irradiation, it is now possible to examine biological effects of intermittent but prolonged exposure to intense irradiation without confounding by such stress. Data from traditional laboratory studies of birth defects arising from exposure of the pregnant animal to microwave irradiation are confounded, but to an unknown extent by psychogenic stress; accordingly, work is being initiated based on the self-selection technique to gain a better purchase on the role of microwave irradiation *per se* in the induction of birth defects.

PESTICIDES AND HERBICIDES

Pyrrolizidine alkaloids (PAs) are produced by plants and have resulted in toxicity and death following their consumption by humans or by grazing animals. Monocrotaline (MCT) is one such PA which produces lung injury and pulmonary hypertension presumably through hepatic metabolism of MCT to a toxic pyrrole (MCTP). Adult Sprague-Dawley rats were treated once with 105 mg/kg MCT subcutaneously or an equivalent volume of isotonic saline and examined 2, 5, 10 and

14 days later. The earliest changes observed were in the platelet count, which was decreased in the MCT animals at 2, 5, and 10 days post injection. Clearance of perfused 5-hydroxytryptamine, a function of pulmonary vascular endothelium, was unaltered in isolated lungs of treated rats until five days after dosing, but decreased progressively thereafter in the MCT animals. The magnitude of this effect was dose related. Inflow perfusion pressure was elevated in perfused lungs of MCT treated animals at day 14. Right heart hypertrophy was also not evident until 14 days after treatment. To avoid the complications of the necessity of hepatic metabolism of MCT, chemically synthesized MCTP was injected into the tail vein to produce similar cardiopulmonary effects. Lung slices from MCT or MCTP treated rats are impaired in their ability to accumulate 5-hydroxytriptamine or paraquat; however, when MCTP was added directly to lung slices, no effect on the accumulation of these compounds was observed. These results suggest that lung cell injury requires more than just direct interaction of MCTP with lung tissue components and raises the possibility that other factors (e.g., circulating platelets, white blood cells, etc.) not present in the isolated tissue are mediating the damage.

The immunotoxicology of organophosphate insecticides was studied. Parathion suppressed the humoral immune response to sheep erythrocytes (SE) in both inbred and outbred male mice. The degree of impairment was more severe in the inbred (C57Bl/6) mice. Immune suppression occurred only at doses which produced moderate to severe cholinergic poisoning. Similarly parathion also produced a 45% suppression of the IgG response. However, the IgG suppression occurred only when parathion was given 6 days post immunization of SE (not when given 2 days post SE). When the immunosuppressive dose was given in 4 equally divided doses, no cholinergic signs occurred and no IgG suppression occurred in either strain. This suggests that the immunosuppression induced by parathion was not due to a direct effect of parathion on humoral immunity, but rather was secondary to the physiological stress associated with the parathion induced cholinergic crisis. This is compatible with the fact that parathion doses which did not produce signs of cholinergic poisoning did not cause significant changes in spleen/body weight ratios or spleen cellularities. When additional organophosphates were tested, suppression of the IgM response was detected after malathion, EPN, Fenitrothion, Abate and methylparathion, but not after DDVP. The latter caused a much less intense cholinergic crisis. Furthermore, the other organophosphates varied greatly in the apparent selectivity of their action on the IgM response. These findings are significant to the field of immunotoxicology. They suggest that toxic chemical stress may play an important role in the immunosuppression detected after organophosphate treatments and that some organophosphates may have a selective (possibly stress-independent) action on the immune system.

The effects of prenatal exposure in utero to the organophosphate, methylparathion (MP) on the neurochemical and behavioral development of rats is being studied. During the second year of the project, studies focused on alterations in protein synthesis and on behavior of the rats after treatment in utero. Methylparathion was administered once daily from day six of gestation until parturition at doses of 1.0 and 1.5 mg/kg, P.O. On days 15 and 19, the incorporation of L-14-C-valine (5 uCi/mmole/100 g rat, s.c.) into protein of embryo, placenta and maternal tissues was determined. There was a dose-dependent inhibition of protein synthesis in maternal and embryonal/fetal tissues in pregnant rats treated with MP. Litters exposed to doses of MP ranging from 0.1 to 1.5 mg/kg were evaluated for ability to perform a variety of behavioral tests. Performance in a battery of tests during the pre-weaning

period including surface righting, negative geotaxis, auditory startle, visual placing, swimming and open field behavior was not different in control and MP exposed pups. Exposure to the 1 mg/kg dose of MP in utero, likewise caused no alterations in shuttle box avoidance, passive avoidance, or rotarod/performance during the post-weaning period. Similar studies are in progress using the 1.5 mg/kg dose of MP. The only observed behavioral alteration associated with MP exposure was a slowed cage emergence behavior. Incomplete data from neuropathological study of 28 day old pups exposed to 1.0 mg/kg MP showed a trend to higher cell counts in brains of OP treated animals.

The trans isomer of (1R,S) permethrin (t-per) was > 110 times more toxic to rainbow trout than to mice by both intravenous and intraperitoneal administration. The importance of trans permethrin biotransformation in this differential toxicity was assessed by measuring rates of t-per biotransformation in trout and mouse tissues in vitro and the effect of inhibitors of drug metabolism on t-per lethality in both species. A previous study had shown that ester hydrolysis by trout liver, plasma and kidney is much slower than that seen in these same tissues in mice. The present work further indicates that oxidation of t-per is 35 times slower in trout liver microsomes than mouse microsomes when the tissue suspensions were incubated at the body temperature of trout and mice (12$0^{o}$ C for trout and 37^{o} C for mice). Inhibition of esterase activity with tri-o-tolyl phosphate (TOTP) produced no potentiation of t-per lethality in trout while the same compound potentiated t-per lethality at least 1.5 fold in mice. Peperonyl butoxide (PIP) alone produced no potentiation in mice but slightly increased t-per toxicity when administered in conjunction with TOTP. PIP caused a slight increase in t-per lethality in rainbow trout but no increase in t-per lethality from control was observed when trout were pretreated with both TOTP and PIP. When drug metabolism was inhibited t-per was still 65 times more toxic to trout than to mice. The data indicate that trout, in addition to hydrolyzing t-per slowly, also oxidize the compound considerably slower than mice in vitro. Potentiation of t-per lethality by TOTP suggests ester hydrolysis to be an important t-per detoxification reaction in mice but not in trout. However, since t-per was 65 times more toxic to the trout than mouse when drug metabolism was inhibited, other factors, such as differences in target organ sensitivity may be involved in the differential toxicity of permethrin.

To investigate translobular behavior of organophosphates as a basis of their macromolecular interactions, a simulation of the liver in vivo was needed. Therefore, a system has been developed to allow recirculating perfusion of the rat liver in situ with the autologous blood only slightly diluted with Waymouth's medium. Analyses with this system have shown that parathion and paraoxon undergo chromatographic translobular migration (CTM) with retention times of 4-6 min and 1-1.5 min, respectively. The system also provided evidence that CTM is not limited to these organophosphates, and extends to other chemicals including naphthalene, 4-nitroanisole, 1,2-dichlorobenzene and 1,3-dichlorobenzene. Thus, it was concluded that the liver functions as a "chromatographic column" in vivo towards certain xenobiotics. Since this phenomenon makes it more important to understand the biochemical heterogeneity of hepatocytes, efforts are being continued to separate subpopulations of hepatocytes.

The herbicide paraquat, although comparatively safe as used agriculturally, has caused over 400 deaths from accidental and suicidal ingestions. Less than a spoonful of the 21% agricultural concentrate leads to death in one to three

weeks. Since 1975, paraquat has been of concern relative to its use in marijuana eradication programs. Regardless of the contact route, the lung is the primary injury site and death may result with pathology similar to that seen from hyperbaric oxygen poisoning. Paraquat can redox-cycle in aerobic tissues to produce the paraquat radical and accumulate oxygen radicals. The molecular mechanism of toxicity is unproven, there is no known effective antidote and therapy consists of measures to remove paraquat from the body. The thesis is that paraquat poisons cells *via* mechanisms we have discovered for oxygen poisoning. Using bacteria as a model at the cellular and subcellular level and in preliminary tests with rats. It was found that like hyperoxia, paraquat poisons specific enzymes required for amino acid biosynthesis and induces stringency. Stringency is a control mechanism which, *via* the regulatory compound guanosine tetraphosphate, leads to rapid cessation of many metabolic pathways. Subsequently, if amino acids are provided to prevent the above consequences, thiamin and niacin become limiting for growth. Cellular thiamin decreases for unknown reasons. Without niacin supplementation, the pyridine nucleotide coenzymes decrease in concentration because of poisoning of the biosynthetic enzyme quinolinate phosphoribosyl transferase, and growth ceases. In preliminary experiments, niacin was beneficial to paraquat-poisoned rats. Nitrofurantoin and to some extent doxorubicin appear to share these toxicity sites.

One study is concerned with the mechanism of action of pesticides on ion transport in lipid membranes. Using membrane permeable probes, it was shown that 3-phenylindole, an antimicrobial compound, alters membrane permeability to cations as much as by several orders of magnitude, and only slightly deceases permeability to anions. It is suggested that the effect is predominantly electrostatic, due to 3-phenylindole molecules oriented at the membrane/water interface. To explain the charge asymmetry of 3-phenylindole action, a membrane patch hypothesis was formulated according to which the membrane contains, in the presence of 3-phenylindole, patches or domains of altered membrane permeability properties.

ORGANIC CHEMICALS

An *in vitro* model, based on isolated hepatocytes in suspension, has been developed for study of rapid early pathological effects of toxigenic halogenated hydrocarbons. By use of this system it has been shown that early suppression by CCl_4 of movement of liver triglycerides to the suspension medium does not require external calcium ions. The significance of this line of work is that it points to a redistribution of intracellular calcium as a possibly significant pathological change. Availability of the isolated hepatocyte experimental system is proving useful in continuing work on this problem.

The biologic and toxic effects of polychlorinated biphenyls are remarkably dependent on their structure. The most active congeners are substituted in both *para* positions and at least two *meta* positions and are approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The addition of one or more *ortho* chloro substituents to those PCBs defined by the above rules tends to diminish their aryl hydrocarbon hydroxylase (AHH)-inducing activity but not necessarily eliminate this biologic effect. The PCB-isostereomers of TCDD and their mono-*ortho* analogs have been shown to exhibit biologic and toxic effects typical of the toxic halogenated aromatics. These PCB congeners all competively displace radiolabelled TCDD from the hepatic cytosolic receptor protein from

male rats, induce AHH activity in male rats and in rat hepatoma H-4-II-E cells in culture. Moreover, there was an excellent correlation between the binding avidities of the individual PCB congeners and their activities as AHH inducers in vivo and in vitro. Preliminary studies with 2,3,4,4',5-pentachlorobiphenyl (PCBP) a prototype of the active mono-ortho-chloro substituted PCBs, also confirmed their TCDD-like activity in the C57BL/6J responsive and DBA/2J nonresponsive mice. In the former strain PCBP induces AHH and causes a 50% reduction in thymus weight six days after administration whereas this compound does not induce AHH nor cause thymic involution in the non-responsive DBA/2J mice. Studies are in progress to further determine the biologic and toxic effects of PCBs and to elucidate their mechanisms of action.

The structure-activity relationship between halogenated polycyclic hydrocarbons and the iron transport mechanism in the duodenum of rats has been more fully defined. Various polychlorinated congeners of polychlorinated biphenyl (PCB) have been tested and the results obtained are consistent with prior findings which relate the induction of aryl hydrocarbon hydroxylase activity (AHH) to the site of the chlorine substitution on the biphenyl ring structure. Concomitant with the stimulation of iron transport in the intestine, preliminary data suggest that intestinal peptidase and sucrase activity are stimulated in more distal portions of the intestine. A colony of mice with sex-linked anemia (sla) has been established from breeding stock obtained from the Jackson Laboratory in Maine. Sufficient animals should be available soon to test whether the iron transport mechanism in the intestine of these mice can be stimulated by a dose of polyhalogenated hydrocarbon. The serosal transfer step of the active transport mechanism for inorganic iron in the intestine of sla mice is defective whereas the mucosal uptake step is normal. The response of sla mice to known polyhalogenated hydrocarbon stimulators of iron transport will provide evidence as to whether an important control mechanism for the active transport of iron may be related to genetic factors. The latter experiments and the structure-activity studies described previously, will more specifically characterize the properties of intestinal iron absorption and further delineate one of the intestinal mechanisms which regulates iron absorption and, thereby, total body iron.

PCBs were shown to produce the same symptom complex in the chick embryo as they produce in adult chickens and other species. A single dose of 3,4,3',4' tetrachlorobiphenyl (TCB) injected into 9 day old embryos causes increased mortality, decreased thymus weight and an increased incidence of pericardial edema in embryos examined at 18 days. The degree of toxicity is dose related. Coincident with the production of toxicity, 3,4,3',4' TCB induces cytochrome P-448 mediated mixed function oxidases as evidenced by increased liver and lung 7-ethoxyresorufin deethylase activity. The toxicity was decreased by the prior administration of benoxaprofen, a nonsteroidal antiinflammatory agent which inhibits both cyclooxygenase and lipooxygenase. The toxicity was decreased without any concomitant decrease in mixed function oxidase induction. The decrease of toxicity by benoxaprofen appears to be the first report of pharmacologic protection against generalized PCB toxicity. The findings have two significant implications: (1) They suggest that products of arachidonic acid metabolism may cause some of the manifestations of PCF toxicity and (2) Since the toxicity can be modified without altering the induction they suggest that the toxicity is not caused directly by the induction.

A research program is aimed at an evaluation of embryotoxic interactions between polychlorinated byphenyls (PCBs) as the primary chemical insult to embryonal tissues followed by a well established teratogen as a secondary insult is underway. Two model teratogens, cyclophosphamide (CPA) and 5-fluorodeoxyuridine (FUdR) are employed for these experiments. The combination of PCBs (in the diet for 90 days prior to breeding = chronic or beginning on day six of gestation = acute) with a single injection of teratogen 260-265 hrs. after mating has revealed several striking results. With respect to FUdR, the most notable interactions with PCBs were found in bone development. While in acute PCBs offspring, the magnitude of the slight long bone reduction caused by FUdR alone was enhanced by PCBs. This did not occur with CPA. In addition, there were also a variety of changes in the expression of embryotoxicity related to paw development. For example, syndactyly in the hind limbs of all fetuses was increased in the acute, but not in the chronic, PCBs group.

The structure-activity relationship of polychlorinated biphenyl (PCB) isomers in the induction of hepatic xenobiotic metabolizing enzyme activities was studied in responsive and nonresponsive mice. PCB compounds usually enhanced the ethoxycoumarin O-deethylase activity more than that of the aryl hydrocarbon hydroxylase of the polysubstrate monooxygenases. Only 3,4,3',4'-tetrachlorobiphenyl increased the aryl hydrocarbon hydroxylase activity to a level resembling TCDD in the induction property. Hexachlorobiphenyls were the most potent inducers of epoxide hydrolase and UDP glucuronosyl-transferases but generally no structure-induction relationship could be established. Polychlorinated paraffins (PCP) were compared as inducers of biotransformation enzymes to polychlorinated biphenyls (PCB) and naphthalenes (PCN), which compounds the paraffin derivatives can replace in industry. PCN compounds proved to be even more potent inducers than PCB compounds but PCPs were very weak inducers in either liver, kidney or intestine suggesting that aliphatic chlorinated hydrocarbons do not contain the structural features which make organochlorine compounds potent inducers of drug metabolizing enzymes.

Several squamous cell carcinoma (SCC) lines were surveyed for their response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The cells were grown in the presence of a lethally irradiated feeder layer of mouse 3T3 cells in Dulbecco-Vogt modified Eagle's medium supplemented with 5% fetal calf serum. Addition of varying concentrations of TCDD (10^{-11} to 10^{-7}M) to confluent cultures of SCC 4,9,13 and 15 cells resulted in a dose-dependent increase in 7-ethoxycoumarin O-deethylase (ECOD) activity with an ED_{50} ranging from 1 to 3 nM. Near maximal induction was observed within 24 hr. Under the same culture conditions, addition of TCDD to SCC 12F2 cells at concentrations up to 1 uM produced no significant increase in ECOD activity. The structure-activity relationship for the induction of ECOD activity in the four responsive lines (SCC) 4,9,13 and 15) showed all the same stereospecificity required for binding to the TCDD receptor in murine liver. Using sucrose density gradient analysis, specific binding of radio-labeled TCDD was detected in the cytosol fractions from the responsive cell lines, but not in the non-responsive SCC 12F2 cells. These data are the first demonstration of the TCDD receptor in human cells.

Studies have focused on benzene metabolism in hepatic microsomes from control, phenobarbital (PB), benzene, and beta-napthoflavone (BNF) induced rats. One form of mixed function oxidase appears to metabolize benzene in control, BNF and benzene induced microsomes at all benzene concentrations, and in PB induced microsomes at benzene concentrations of 0.8 mM or below. This enzyme is

increased by benzene pretreatment; activity in BNF induced microsomes is similar to control microsomes. A different enzyme acts in PB induced microsomes at benzene concentrations above 0.8 mM. The two forms differ in kinetics, pH optima, and sensitivity to fluoride and the inhibitor metyrapone. All microsomal preparations form phenol and an unidentified aqueous metabolite from benzene. The aqueous metabolite is extremely polar, but it is not an anion. This aqueous metabolite is also formed from phenol in rat liver microsomal incubations, but is not formed from phenol with either boiled microsomes or omission of a NADPH generating system. Formation of this unknown compound is induced by phenobarbital pretreatment and inhibited by metyrapone, further suggesting that cytochrome P450 is involved. When phenol is incubated with benzene, the production of the aqueous metabolite from benzene is decreased with a slight increase in the amount of phenol coming from benzene.

It has been found that alcohol treatment of rats increases the hepatotoxicity of carbon tetrachloride and increases specifically the biotransformation of carbon tetrachloride to phosgene. These results are significant in that they implicate factors other than lipid peroxidation, specifically phosgene formation, in carbon tetrachloride toxicity. The effect of hypoxia on carbon tetrachloride toxicity was also studied. It was found that exposure of rats to carbon tetrachloride in a hypoxic environment (12% oxygen) increased markedly the hepatotoxicity of carbon tetrachloride. This increase in toxicity was accompanied by an increased covalent binding of carbon tetrachloride metabolites to hepatic microsomal lipids and proteins indicating that critical tissue alkylation may be involved in liver damage. A particularly important finding was the synergistic hepatoxicity of carbon tetrachloride and chloroform. These studies grew out of the earlier studies on the mechanism of potentiation of carbon tetrachloride hepatotoxicity by alcohols. When subthreshold doses of carbon tetrachloride and chloroform were given together to rats, significant hepatotoxicity was produced and the resulting liver damage was accompained by increased liver calcium and triglyceride concentrations. Because toxic chemicals are usually encountered in mixtures, these findings suggest that toxic interactions must be considered in assessing the potential toxicity of chemicals.

The metabolism of vicinal-dihaloalkanes was investigated. Stereo chemical studies showed the meso-1, 2-dideutero-1,2-dichloroethane was metabolized to 1,2-dideuteroethylene. This finding demonstrates that the metabolism of vicinal-dichloroethanes proceeds through the formation of an intermediate episulfonium ion. Such ions are potent electrophiles and have been implicated in the mutagenicity of vicinal-dichloroethanes. The enzymatic reduction of alphahaloketones has also been studied. The actual substrate for the enzyme is the S-phenacylglutathione compound formed by the chemical reaction of glutathione and phenacyl halides. The enzyme has been purified about 300-fold, has a molecular weight of about 39,000 daltons, is an acidic protein (pI - 4.5), and is antigenically distinct from glutathione S-transferases. Studies on the substrate specificity of the enzyme are presently in progress. Other studies have dealt with the enzymology of cysteine conjugate betalyase which cleaves S-substituted cysteines to yield thiols, pyruvate, and ammonia. This enzyme may be involved in the production of nephrotoxicity by halogenated alkenes. In the kidney, the enzyme is located in both the cytosolic and particulate fractions. Current studies are aimed at the purification and characterization of the enzyme.

Studies indicate that trichloroethylene oxide is not a chemically or catalytically important intermediate in the enzymatic oxidation of the cancer suspect trichloroethylene to chloral. To account for these observations, it is proposed that an oxygenated cytochrome P-450-trichloroethylene complex is formed which can rearrange to give the known metabolites. Trichloroethylene metabolism was studied in mouse and rat liver microsomes and isolated hepatocytes and in reconstituted enzyme systems containing eight different purified isozymes of rat liver cytochrome P-450. These studies strongly suggest that trichloroethylene oxide is not the trichloroethylene metabolite responsible for irreversible binding to DNA and protein. In studies with isolated hepatocytes, the reactive metabolites were shown to be stable enough to leave the cells before binding to protein and DNA. Further, binding was greater in mouse than rat systems, in line with previously reported differences in rodent carcinogen bioassays. More than 97 percent of the metabolism of the cancer suspect acrylonitrile in isolated rat hepatocytes can be accounted for in terms of pathways that have previously been described. The primary modes of metabolism include conjugation with glutathione to form S-(2-cyanoethyl)-glutathione, binding to cysteinyl groups of protein, and oxidation to 2-cyanoethylene oxide, which hydrolyzes to release cyanide which is converted to thiocyanate. Some irreversible binding to DNA can be detected, although the level is no more than 0.5 percent of that of protein binding. As in the case of trichloroethylene, adducts are primarily formed with extracellular DNA. Cyanide is rapidly converted to thiocyanate and does not accumulate, and cyanide does not play a role in cell death. In order to study the metabolism of the cancer suspect vinylidene chloride, methods were developed for the synthesis of 1,1-dichloroethylene oxide and its rearrangement and hydrolysis products determined. The enzymatic metabolism of vinylidene chloride gives rise to dichloroacetaldehyde, which cannot be explained in terms of the epoxide as an intermediate. These and similar studies should present a better understanding of the mechanisms of metabolism and modes of action of potentially dangerous substituted olefins and provide a scientific basis for risk assessment.

BIOMECHANISMS

Nitropolynuclear aromatic hydrocarbons have been detected in various environmental samples. These agents are highly mutagenic in S. typhimurium assay, but little is known about their carcinogenic activities and particularly their mechanisms of action in mammalian systems. Two compounds were selected, 5-nitroacenaphthene (5-NA) and 1-nitronaphthalene (1-NN), to compare their metabolic transformations by rat liver. 5-NA was found by HPLC in airborne particulates and in diesel emission exhaust and is carcinogenic in both rats and mice. In contract, 1-NN showed no carcinogenic effect in either species under the same conditions. The mutagenic activity of 5-NA in both S. typhimurium TA 100 and TA 98 was much greater than that of 1-NN. The metabolism studies of both 5-NA and 1-NN were performed under the same conditions, either aerobic or under 10% O_2 in N_2 atmosphere. Under aerobic conditions 5-NA gave metabolites from both nitro reduction and C-hydroxylations. In contrast 1-NN under identical conditions gave mainly metabolites produced from ring hydroxylations; nitro reduction was not observed. The facile nitro reduction of 5-NA as compared to 1-NN may be reflected in the high mutagenic activity and possibly the carcinogenic activity of the former compound. Since the nitro reduction pathway is important in the metabolic activation of this class of compounds and oxygen is known to inhibit it, the metabolism under reduced amounts of oxygen (10% O_2 in N_2) was studied. The results suggest that even under reduced amounts of oxygen,

nitro reduction is more facile in 5-NA than in 1-NN. In view of the widespread industrial usage and environmental presence of nitro aromatics and the lack of information on their mechanism of action, the results of this study are essential in evaluating the actual risks of human exposure to these agents.

Highly sensitive methods are required for the quantitation and kinetic analysis of repair of carcinogen modified DNA components. This becomes most important for the in vivo detection of low levels of DNA modifications and when using small numbers of cells. Antibodies have been developed against O^6-ethyldeoxyguanosine, a potent precarcinogenic lesion in DNA. The specificity of these antibodies has been determined using enzyme-linked immunosorbant and radioimmunological assays. Other techniques such as fluorescent linked immunoassays and approaches using monoclonal antibodies and antibodies directed to precarcinogenic lesions like pyrimidine dimers and N^7-alkylguanosine are underway. These antibodies are being used to quantitate DNA damage in vivo and in vitro. Thus, immunological detection of DNA damage appears to be a very important and sensitive approach to determining the initial and molecular consequences of carcinogens.

Studies to characterize methods for in vitro activation of promutagens by plants to compare the activities of promutagens assayed under conditions of plant activation with activities found in mammalian liver microsomal fractions (S-9) and to compare in vitro and in vivo mechanisms of plant activation are underway. During the past year several techiques were evaluated separately and together for their ability to produce the most efficient plant homogenates using both genetic (Salmonella) and biochemical endpoints. It was discovered that common enzymatic protectants such as ß-mercaptoethanol inhibit expression of his^+ Salmonella revertants. Since enzymatic protection of plant homogenates is essential to their activity, a preliminary search was conducted for a protectant compatible with the Salmonella assay. Leupeptin (0.001 mM) appears to be a suitable protectant. The mutagenic potential of the presence of ^{14}C and ^{3}H labels in nonmutagenic compounds was investigated and the potential comutagenicity of these isotopes was compared with known mutagens to determine their compatibility with the Salmonella assay. The presence of these isotopes did not induce non-mutagenic compounds to become mutagenic nor cause a comutagenic effect with sodium azide. These data suggest that radiolabelled compounds can be used to follow and identify metabolites of promutagens under conditions of either plant or animal activation.

Rat liver microsomal vesicles actively sequester calcium ions. The depression in this capacity resulting from the action of liver poisons probably plays an important role in the attendant cell injury. A new method, based on steady-state isotope exchange of $^{45}Ca^{2+}$ has been developed to study the kinetics of calcium sequestration. The significance of this new method is that it will permit detailed study of mechanisms by which various hepatotoxins depress the calcium sequestering property of the microsomes.

Purification methods to isolate eight different isozymes have been further developed in rat liver cytochrome P-450 in electrophoretically homogeneous form and these cytochromes have been characterized using spectral, electrophoretic, and catalytic methods. Methods to immunologically quantify all of these isozymes in crude tissue samples have also continued to be developed. These immuno-chemical assays were used to quantify the concentrations of the eight

individual cytochromes P-450 in liver microsomes of untreated rats, rats treated with commonly used inducing agents, and a series of ten polybrominated biphenyl congeners. The results show decreases of certain isozymes for the first time and that levels of all eight forms of cytochrome P-450 are independently regulated. These samples and quantitations were also used to establish the usefulness of the various metabolites of the anticoagulant warfarin as markers of the function of many of the individual cytochrome P-450. These immunochemical techniques were also used to study the species comparison and tissue distribution of another enzyme, microsomal flavin - containing monooxygenase. Immunochemical techniques and warfarin metabolism profiles were also used to gain insight into the similarities and differences among cytochrome P-450 in rats and mice. Antibodies to cytochromes P-450 have also been used to examine the selective localization of the different cytochrome P-450 isozymes in rat liver, pancreas, skin, lung, and other tissue. Progress has been made in the development of more monoclonal antibodies to rat liver cytochromes P-450 to further increase the specificity of these immunochemical procedures. A series of studies with cyclopropyl substituted amines and ethers led to the view that cytochrome P-450 metabolizes heteroatomic compounds via stepwise one-electron oxidations at the heteroatom. These studies and others with vinyl halides have led to the proposal of a unified mechamism for the catalytic action of cytochrome P-450, in which all known oxidative reactions can be explained by abstraction of an electron or hydrogen atom by a perferryl iron-complex. Recent studies on the iron spin state have shown that the presence of high spin iron is a very poor indicator of catalytic function, in contrast with existing dogma.

Dietary components proved to be major determinants of polysubstrate monooxygenase activities. Also, ethanol, when given in drinking water to rats, caused marked microsomal membrane changes in the liver and intestinal mucosa by increasing the amount of microsomal phospholipid contents and simultaneously increasing the saturation degree of phospholipid fatty acids which was reflected in the binding of fluorescent probes to membranes. Both the hepatic and intestinal cytochrome P-450 concentrations and ethoxycoumarin O-deethylase activities increased while no changes were present in the aryl hydrocarbon hydroxylase activities. Simultaneous dietary riboflavin deficiency drastically decreased both the ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase activities while fewer changes were observed in the epoxide hydrolase and UDP glucuronosyltransferase activities suggesting the functional role of riboflavin in the regulation of the monooxygenase activities. Dietary cholesterol 2% in the diet) had major effects on the hepatic and intestinal biotransformation activities and membrane compositions. The simultaneous exposure to xenobiotics further modified the response. In addition, the dietary cholesterol increased the hepatic and intestinal microsomal cholesterol content and also modified the microsomal phospholipid fatty acid composition. The cytochrome P-450 levels were about 2-fold in the livers of rats having cholesterol in their diet in comparison with their cholesterol-free fed counterparts. The inducibility of cytochrome P-450 by phenobarbital was present only when the 2% cholesterol diet was given. The inducibility of individual polysubstrate monooxygenases was dependent on the dietary cholesterol content suggesting that the dietary cholesterol possibly has cytochrome P-450 subspecies-dependent regulation power over the monooxygenase activities. As the dietary cholesterol proved to be an extremely potent modifier of both the hepatic and intestinal xenobiotic metabolisms, further studies are warranted in the evaluation of the role of dietary cholesterol and other lipids in the possible metabolic activation or carcinogenic/toxic compounds.

The mechanism(s) by which the hepatotoxin allyl alcohol acts selectively on periportal rather than pericentral cells is being investigated. A method is currently being developed to measure periportal and pericentral rates of allyl alcohol metabolism. Infusion of allyl alcohol into perfused livers from fed, phenobarbital-treated rats increases NADH fluorescence similarly in both periportal and pericentral regions of the liver lobule detected with a micro-light guide. Infusion of methylpyrazole, an inhibitor of alcohol dehydrogenase, abolished the increase in NADH fluorescence. When the concentration of allyl alcohol infused into the liver exceeded 200 uM, a 30 to 40% inhibition of oxygen uptake was observed. Infusion of methylpyrazole also prevented the inhibition of oxygen uptake while infusion of acrolein, the metabolic product of allyl alcohol metabolism, inhibited hepatic respiration. Allyl alcohol also decreased cellular ATP and the ATP/ADP ratio and increased malate and citrate. Rates of glycolysis and glycogenolysis were also increased by allyl alcohol. Local rates of oxygen uptake were measured using mini-oxygen electrodes placed on periportal and pericentral regions of the liver lobule. Infusion of allyl alcohol caused a 30% inhibition of respiration in periportal regions but had no effect on oxygen uptake in pericentral areas. Data indicate that although metabolism of allyl alcohol occurs at similar rates in both periportal and pericentral regions of the liver, allyl alcohol inhibits respiration selectively in periportal regions. This finding may explain why allyl alcohol selectively damages periportal regions of the liver lobule.

The chemical mechanisms underlying the cytochrome P-450 mediated oxidative metabolism of halocarbon compounds, many of which pose significant threats to human health, is under investigation. Such mechanistic understanding is essential to developing the ability to predict the biological outcome of metabolism of a halocarbon substrate, since metabolism by cytochrome P-450 may result either in inactivation of a toxic compound to an innocuous one or to bioactivation of an inactive one to a proximate toxin or carcinogen. Thus, the biological outcome of metabolism is a function of the spectrum of metabolites, which is a consequence of the substrate structural features and the chemical mechanism and pathway of metabolism. It was demonstrated that halogen containing compounds undergo alpha-hydroxylation and halogen oxygenation processes in a fashion analogous to their heteroatom counterparts (nitrogen, oxygen, phosphorus, sulfur). In order to assess the extent and toxicological impact of the alpha-hydroxylation and halogen oxidation pathways, the metabolic products resulting from the cytochrome P-450 mediated metabolism of the propyl halide series (chloride, bromide, iodide) were determined with attention directed at the influence of the halogen substituent in the halide series on the distribution and types of metabolic products. Metabolic products derived from both types of reaction were observed with qualitative rates of cytochrome P-450 mediated disappearance of the propyl halides following the order: propyl iodide > propyl bromide > propyl chloride. In additional studies, the metabolism of the carcinogen, ethylene dibromide, bis-4'-chlorophenyl-1,1,1-trichloroethane (DDT), and of 1,1,1-trichloroethane (methylchloroform) were examined with efforts directed at determining the total metabolite profiles, including the chemical nature of the adducts bound to protein and DNA. Significantly, the mechanism of both alpha-hydroxylation and heteroatom oxygenation appears to proceed via initial single electron transfer from the substrate to the enzyme producing transient, highly reactive intermediate radicals. This suggestion is based on a series of studies with cyclopropyl substituted amines, ethers, sulfides and halides, suggesting that cytochrome P-450 mediated halogen oxidation of organohalides to hypervalent, reactive species (cation radicals,

organohalogen oxides) may constitute a previously unrecognized metabolic process of critical relevance to the toxicology of this chemical class.

BIOLOGICAL AGENTS - TOXINS

Type B botulinum toxins from different culture strains fall into two groups whose toxicities by the intraperitoneal route are (i) about equal for infant and adult mice and (ii) more than 500 times greater for infants. Antigenicities and molecular sizes of the toxins of these two groups are identical. The component H and L subunits of representatives of the two toxin groups were purified and dichains were formed with the complementary (H + L) subunits from the same (=reconstitution) or different toxins (hybridization). Reconstituted dichains had mouse infant to adult toxicity ratios comparable to those of the parent toxins. The hybrid whose H chain was from the parent toxin having a high ratio had correspondingly high ratio, but the hybrid having H subunit of the other parent had a ratio close to one. The high infant toxicity ratio was the property of the H chain; origin of L chain in the hybrids had little effect on toxicity ratio. Hybrid dichains did not form spontaneously in mixtures of complementary subunits from different botulinum toxin types but a preliminary trial suggested this might occur in a hybridization procedure using sulfonated L chain.

Important progress has been made describing some of the general features of microbial S-conjugation of pollutants, heretofore, a virtually unknown area of environmental toxicology. The first definitive characterization of microbial glutathione transferases, isolated from three sources, including a protozoan, Tetrahymena thermophilia, a yeast, Candida lipolytica, and an alga, Euglena gracilis were carried out. These findings imply a widespread distribution of such detoxification enzymes in microbial ecosystems, and raise the interesting possibility that such processes lead to the excretion of new organosulfur metabolites into the environment.

Caffeine, by mechanisms not well understood, inhibits DNA repair and recovery processes in mammalian cells exposed to mutagens and carcinogens. Among the biological consequences attributed to this inhibition are enhancement of chromosomal damage and lethality; an increase or decrease in induced mutations, maligant transformation and teratogenicity, and a decrease in induced carcinogenicity. Evidence is accumulating in microorganisms and mammalian cells that the metabolism and availability (pool size) of DNA precursors play a critical role in DNA repair and the mutagenic and cytotoxic actions of many environmental agents. Genetically and biochemically well characterized purine and pyrimidine auxotrophic mutants of Chinese hamster ovary cells (CHO-K1) were used to investigate the effects of caffeine on DNA precursor metabolism. Caffeine effectively inhibits both the de novo synthesis and reutilization of exogenous purines, the former prior to the third step of the pathway and the latter seen as inhibition of conversion of hypoxanthine, guanine and adenine into their respective di- and triphosphates. All these results are consistent with the hypothesis that caffeine-enhancement of lethality is due, at least partially, to perturbations in DNA (repair) synthesis resulting from interference with DNA precursor metabolism. PRPP appears to be a key molecule.

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