ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Fiscal Year 1981

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1981 ANNUAL REPORT

October 1, 1980 through September 30, 1981

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OFFICE OF THE DIRECTOR

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Summary Statement

An event of considerable significance during the year was the approval by the Secretary, DHHS on July 14 of a major reorganization within the Institute. This reorganization came about as a logical outcome of events that began in 1978 with the establishment of The National Toxicology Program. The action established four distinct program areas within the NIEHS, namely: (1) the Toxicology Research and Testing Program (TRTP); (2) the Biometry and Risk Assessment Program (BRAP); (3) the Intramural Research Program (IRP); and (4) the Extramural Program (EP).

Specifically, the reorganization (1) establishes the TRTP within the Institute to perform toxicology test research and development, test validation, and the limited conduct of tests as assigned to NIEHS under the NTP; (2) abolishes the National Cancer Institute's Bioassay Program within the Division of Cancer Cause and Prevention and transfers those functions and staff to NIEHS. (Staff will be located both at the NIH in Bethesda, Md. and at NIEHS in Research Triangle Park, N.C.); (3) establishes the Biometry and Risk Assessment Program within the Institute to fully recognize the expanding role of the former Biometry Branch which pursues research and contributes support and expertize in computer, statistical, mathematical, and epidemiological areas, emphasizing the increased importance of risk assessment to the Institute's mission; (4) establishes in the Office of the Director, the Office of Facilities Engineering responsible for building operations, maintenance, and other support services, and (5) abolishes the Research Resources Program and transfers the functions of the Research Services Branch to the Office of Facilities Engineering; moves the Comparative Medicine Branch and the Laboratory of Environmental Chemistry to the IRP and transfers the Environmental Biology Branch to TRTP.

This reorganization provides for a closely integrated and coordinated response to the challenges and requirements of the National Toxicology Program, emphasizing the Institute's commitment to chemical test development and risk assessment while providing the realignment within the Intramural Research Program necessary to meet the challenges in the years ahead. Intramural Research changes have put greater emphasis on chemistry and pharmacology intensifying investigations on the toxication-detoxication process of environmental agents within biological systems.

During the year the Institute also realized a long awaited objective to occupy its new laboratory/administrative building in Research Triangle Park. Early in April, the immediate Office of the Director and his staff along with many of the Institute's top administrators and their staff, the budget office, and the Institute's computer operations moved into administrative modules A and B of the 5-module facility. These moves which left vacancies on the Institute's North Campus in Research Triangle Park allowed the Extramural Program office to be relocated from off campus rental property back to the North Campus. Much of the construction on the new building has been completed allowing a target date of mid 1982 for total occupancy. At that time the three laboratory modules C, D and E will be occupied by the scientific research staff and a long envisioned Institute goal will then be realized.

The intramural program continues in the forefront of advanced science, providing the data base necessary to build programs of disease prevention for individuals and populations. Recently reported studies from one of the Institute laboratories have shown that diethylstilbestrol normally not a mutagenic agent produces aneuploidy in animal tissue cultures, leading to the development of cancerous transformations in these cells. Since other carcinogens can induce aneuploidy these studies with DES should stimulate new thinking about how some chemicals cause cancer. This work was highlighted in the June 19th issue of Science with a cover picture to accompany the scientific story inside. 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 by Institute scientists to evaluate both nutritional and hormonal requirements for embryonic development and also the systems predictiveness for chemical teratogenesis.

During the year, a battery of tests has been developed for the assessment of neurobehavioral toxicity in rats and mice. The tests, for both younger and older animals, assesses locomotor activity, fore- and hindlimb grip strength, startle responsiveness to an acoustic and air puff stimulus, rotation orientation response (negative geotaxis), tail flick to a thermal stimulus, rectal temperature, and body weight. Learning and retention measures are also taken. Nine representative neurotoxicants and/or pharmacological agents are being profiled in a collaborative research project with Stanford Research Institute.

Current research has focused on acrylamide, a neurotoxicant, which produces a "dying-back" central peripheral axonopathy. The peripheral neuropathy produces by acrylamide in adult animals has been characterized following repeated dosing which produces a progressive distal to proximal weakness of the limbs. Recent studies in adult animals have concerned its possible central effects, while studies concerning the neurobehavioral and neurochemical specificity of acrylamide on dopamine functioning are now in progress.

In an effort to develop an understanding of molecular mechanisms of mutagenesis, both genetic and biochemical approaches are being used. Being studied is the replication of DNA, the basic determinant of heredity in both prokaryotic and eukaryotic systems, while work is also progressing on the mechanisms of mutagenesis using the T4 bacteriophage system. The mutagen testing program tests chemicals for their potential mutagenic properties and is at the same time developing and validating mutagen tests. To meet these two aims contracts with several agencies are designed to test both chemicals of unknown mutagenicity and, in parellel blind studies, control chemicals whose mutagenic properties are known.

The nature of genes and how they function is being examined to gain understanding of mutation events and their impacts on reproduction and development in multicellular organisms. The effect of environmental agents on the genetic apparatus can be fully understood only if the molecular structure of genes and the details of gene activity and regulation are known. Recently the development of techniques for purifying genes and characterizing them in molecular terms has brought some startling developments and promises of answers to some of the most basic questions in genetics.

In another area of genetics research, the mechanism for induction of mutations in mammalian cells in vivo is being studied, with several different selection

and detection mechanisms used for recognizing the mutant cells. A technique has been developed for detecting mouse sperm which reacts with monospecific antibodies to rat LDH-X, indicating the presence of point mutations. This is the first time in history that anyone has detected a type of mutation in sperm which is likely to be point mutation.

Work is also concerned with developing methods for detecting germinal mutations and the consequences of germinal mutations. The purpose is to seek more effective procedures to detect germinal mutations while providing greater understanding of the impact of individual mutations. It is directly concerned with the problem of human genetic health risks caused by environmental mutagens.

Research efforts relating to the respiratory system are concerned with cell differentiation and regulation of cell function of epithelial cells lining the conducting airways. Studies are being carried out in vivo as well as in cell culture systems. One of the cell types currently under investigation is the Clara cell which, in the rabbit seems to be the major secretory cell of the tracheobronchial tree. The cells ultrastructural and cytochemical characteristics, as well as their distribution are presently being determined in both hamsters and rats.

Research efforts are also concerned with neuro-endocrine cells of the lungs as well as with identification of peptide hormones that may be important for the control of various lung functions such as mucus secretion, bronchial and vascular muscle tone. Other studies concern the metabolism and transport of prostaglandins (PG) and related substances in the lungs, with one of the key topics an attempt to identify the factors controlling production of PG and thromboxanes (TX) by pulmonary endothelium which in turn relates to the control of intravascular thrombosis.

Studies concerned with the pathogenesis of asbestosis and other pneumoconioses have been initiated on the early translocation of chrysotile asbestos and silica, following inhalation exposure. Investigators observed that fiber containing macrophages accumulated at alveolar duct bifurcations, which is known to be the site of later pathologic tissue reactions to asbestos. This accumulation of macrophages was most conspicuous at the proximal alveolar duct bifurcations. The early translocation of asbestos fibers to the interstitium, the preferential accumulation of fiber containing macrophages at alveolar duct bifurcations and the long persistence of particle containing macrophages are all thought to be important elements of the pathogenesis of dust related lung diseases.

Pharmacological studies are being carried out to elucidate the relationships between the transformation and translocation of chemicals and toxicity in various target organs and cells of the body. In these studies pharmacological and pharmacokinetic concepts are being used to characterize, in detail, the mechanisms by which environmental contaminants exert biological effects. The Laboratory of Pharmacology serves as a focal point within NIEHS, NIH and DHHS for marine and freshwater biomedical research. In this context investigations concern possible direct impact on human health by contaminants present in the aquatic environment (including drinking water) and accumulated by aquatic animals. One portion of the research can be described as an integrated, multi-faceted effort to understand the role of chemical metabolism in the mediation

of toxicity such as overt tissue damage, or more subtle effects such as carcinogenesis, mutagenesis, teratogenesis.

A major emphasis in marine research focused on toxication-detoxication systems, transport and excretory mechanisms and membrane toxicity. The uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism in the storage and the chemical form of the accumulated xenobiotics in these species is being assessed with major emphasis 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 of higher levels of pollutants than single chemical exposure.

Other research is concerned with defining and measuring reliable dose-response relationships in animals which will provide a basis for estimating the magnitude of risk at specified levels of toxicant exposure. The primary objective is to characterize the most appropriate pharmacokinetic transformations of applied dose for use in the statistical models. Also of concern is the various aspects of hepatotoxicity emphasizing the more subtle alterations in liver function following exposure to environmental agents. Investigators plan 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.

Heavy metal research is oriented toward investigation of the mechanisms by which heavy metals produce subcellular damage using quantitative electron microscopy, biochemical, and physiological measurements, with particular emphasis on understanding the mechanisms by which heavy metals interact with biological processes to effect toxicity in human populations. Specific studies include investigations concerning the mechanisms by which agents such as cadmium produce renal damage and low molecular weight proteinurias.

Problems of intestinal toxicology and function at the cellular, subcellular and molecular level are also being investigated including areas that represent unknowns in the field of intestinal absorption and metabolism that may be related to selective/specific gut toxicoses. 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.

Laboratory investigators have developed wavequide systems for exposing cell preparations which allow stirring during exposure. These systems provide the capability for accurate determination of specific absorption rates and for controlling temperature at any desired level between 10 and 60 degrees celcius. A circularly polarized waveguide system is currently under development that will provide the capability for long-term exposure of animals to 918 and 2450 MHz microwaves while being housed and cared for under near normal animal caretaking conditions. Using Japanese Quail, investigators found that fertility was significantly reduced using matings of exposed males with both exposed and control females while hatchability of fertile eggs was unchanged. Experiments to determine the effects of in utero noise exposure on the conceptus and fetus have continued, with teratogenic effects, lowered pregnancy rate, excess early stage resorption, and lowered maternal and fetal weight were observed.

During the year, the National Advisory Environmental Health Sciences Council reviewed 442 applications assigned to NIEHS as primary or secondary assignee. One hundred and twenty-eight new and competing awards were made; 106 regular research grants, two Environmental Health Sciences (EHS) Centers (competing), two Research Career Development Awards; four Mid-Career Development Awards, and 12 individual NRSAs and two Senior Fellowships. These new and competing awards, plus the non-competing awards, brought the 1981 total awards to 406 active grants, a decrease of 17 awards from Fiscal year 1980. The New Investigator Research Award (NIRA), previously the Young Environmental Health Scientist (YES) Program, has continued to attract young investigators.

Requests for applications (RFAs) have been issued or are being developed in "Extrapolation and Risk Estimation"; "Human Health Effects of PBB"; "Immunotoxicology to Environmental Agents; and "Biological Effects of Chemical Interactions" in an effort to bolster the Institute's research portfolio in special emphasis areas.

The Secretary, DHHS, approved the charter for an Environmental Health Sciences Review Committee. Potential members are being identified and all administrative activities are being completed pending approval to appoint members. Once the Committee is in place, NIEHS will assume responsibility for review of Program Projects in addition to its current grant review responsibilities.

Investigators supported by funds administered by the Institute's Extramural Research Program have added significantly to the knowledge base about the toxicity and the mechanism of toxicity of a host of environmental agents. One of these studies on the effects of metals has found that dietary methyl mercury produced neurological and developmental abnormalities in children exposed *in utero* and this has necessitated the reappraisal of the allowable levels of this compound in foods. Preliminary estimates on the levels of methyl mercury suggests that the fetus may be more sensitive than was previously thought. In another study it was found that a defined high protein, low fat, low residue diet markedly increases fecal mercury excretion after methylmercury exposure, also adult male and female mouse kidneys concentrate mercury differently and this difference is related to testosterone stimulation.

One significant epidemiological study indicated that there was a statistically significant excess of digestive tract cancer among male shoe workers. The results support previous reports of bladder cancer hazards in the leather products industry. The NIEHS supported study suggests an association between work in the shoe industry and cancer of the digestive tract and between work in the leather industry and cancer of the stomach among males. These associations have not been reported previously.

Pulmonary hypersensitivity to industrial chemicals is being studied in a system using guinea pigs as a model. In the study, a plethysmograph is used to study the respiratory rate and tidal volume in sensitized and control animals. The system should allow for complete assessment of the pulmonary sensitizing properties of industrial chemicals.

The metabolism, disposition, and interaction of waterborne pollutants in fish is being studied to determine the persistence and bioaccumulation of several classes of chemicals, the rate at which these compounds are metabolized, and to

gain a better understanding of the role of metabolism and the persistence in bioaccumulation of these hazardous materials in the food chain.

Studies are underway which will be useful in predicting conditions in which non-target vertebrates may be unusually susceptible or resistant to the toxic action of pesticides and other chemicals as a result of exposure to pesticides. Upon repeated exposure to certain organophosphorus acetylcholinesterase (AchE) inhibitors, rats develop tolerance apparently through alterations of cholinergic receptor sites in the brain. Additional studies have shown that the development of tolerance to chronic organophosphorus treatment is at least partly, mediated by a decrease of the muscarinic cholinergic receptors.

The strong correlation which exists between mutagenicity and carcinogenicity encourages development of mutagen-sensitive assays. The development of mutagen-sensitive Drosophila strains defective in precisely defined biochemical steps in DNA-repair processes is expected to be able to provide discriminating tester strains for determining the mutagenic capability and mechanism of action of environmental agents.

In another research project the carcinogenic action of formaldehyde (HCHO) and hydrogen chloride (HCl) including the possible role of bis(chloromethyl) ether (BCME) formed from the interaction of HCHO and HCl are being studied. Rats exposed to lifetime regimes of single and combined chemicals are under observation.

The neurotoxicity of various organic intermediates on the central nervous system of primates is being studied using Acrylamide as the model compound. The studies utilize morphological and electrophysiological techniques to pinpoint the most vulnerable areas of the peripheral and central nervous system and to monitor the degree of recovery in the living animals.

Two assay systems are being explored to test the mutagenic or carcinogenic potential of compounds in the environment. The first system is a host-mediated assay by culturing mammalian cells in diffusion chambers (DC) and implanted into mice. The second system is a liver homogenate mediated assay by incubating DC filled with the rat liver extract, cofactors and a compound under test with mammalian cells in vitro. The two systems have been tested for screening mutagenic and/or carcinogenic potential of environmental compounds especially those that need to be metabolically activated. The systems are simple, sensitive and economical in time and cost.

Dichlorobenzidine (DCB) widely used in the manufacture of azo dyes and as a curing agent in the production of polyurethane foam is known to be a potent carcinogen in animals and therefore assumed to be carcinogenic to man. The metabolic fate and the mechanism by which it produces its carcinogenic effect are under investigation. In addition, binding studies of DCB to polyribonucleotides has indicated that these aromatic amines show a marked preference for reaction with guanine in the experimental animals' genetic material.

The metabolism and toxicity of carbon tetrachloride and benzene, the role of sulfhydryl compounds in carbon tetrachloride toxicity and the mechanism of alcohol potentiation of carbon tetrachloride toxicity is also being investigated through Institute supported research. These studies will help elucidate the mechanism by which chemicals produce liver damage which is a common toxic effect of halogenated solvents.

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

This office is concerned with the evaluation of human health hazards, particularly those from chemical pollution. It is of importance indeed to assess toxicity of newly produced chemicals to protect the public before exposure could have occurred. Often it has been difficult to explain carcinogenic risks to the public, but it is hoped that the misunderstandings can be eliminated.

Hazards from occupational exposures to carcinogens have been focused upon by NIOSH while the NIEHS is concerned with environmental exposures to these and other chemicals. The environmental exposures are less frequent and of lower intensity and therefore this exposure alone would be unlikely to produce a significant incidence of cancer in the population.

At the much lower concentration of exposure, additional factors come into play if cancer is still to occur which may arise from eating, drinking, and smoking habits as well as from vocational preferences. They may present a different kind of exposure -- less readily quantitated and much less easily controlled.

The reason for the difference is shown in animal experiments where at high exposure over a long period of time a large percentage of the animals will get tumors, while at lower levels the susceptible group gets smaller and a level of exposure will be reached that shows no adverse effect -- a level erroneously considered a "no-effect" level. At that level introduction of prolonged treatment with a promoter will still produce tumors. The contributions of initiator and promoter towards carcinogenesis depend on temporal relationships as well as target organ specificities.

A chemical frequently needs to be metabolized to become active as a carcinogen and competing metabolic pathways exist which can be affected by exposure to other environmental factors. Among these are chemicals that act as enzyme inducers. Their dose-level requirement, target organ, and type of enzyme induced are some of the factors which make prediction of health effects from such combined exposures difficult.

An activated carcinogenic chemical will be able to bind to micro- as well as macromolecules. For those that bind to DNA it is quite important to learn how the chemical reacted with the DNA constituents and on what location on the purine and pyrimidine bases. Some products can readily be removed by enzyme action. Inadequacy of repair enzyme activity in certain tissue may lead to persistence of the DNA damage and ultimately may lead to cancer. Such DNA repair enzyme deficiencies exist in certain human diseases, but it has been found that chemicals can also act as DNA repair inhibitors which opened another important area for health hazard assessment of environmental chemicals. This topic is being reviewed in depth, and a paper is being prepared by Ms. Jean Bernheim.

Because of the importance of adverse effects of ingestion of PBBs as had occurred accidentally in Michigan some years ago, staff of OHHA prepared a complete review

of the published findings of PBB toxicity in experimental animals which will be published in EHP.

A subcommittee to the NTP Chemical Nomination and Selection Process Committee continued its activity to search for important chemicals in need of bioassay. All of OHHA as well as Drs. D. B. Walters and B. A. Fowler participate in this activity. Besides the preparation of lists of chemicals with appropriate justification and other required information, attention was paid to the problem of interaction of chemicals with the target organs in producing potentially greater toxicity than anticipated from the action of individual chemicals. The situation at Love Canal may be a prime example of multiple chemical exposures, but also interactions may result from chemical exposures from different sources.

Dr. Posner participated in an Asbestos Substitute Workshop in Washington to be up to date on potentially new developments and hazards which may arise from the use of new materials to replace asbestos from its many uses or to physically change the asbestos fibers in some products to prevent toxicity. He prepared documents on the problem submitted to the NIEHS selection committee. Dr. Piver attended a meeting on metals for photovoltaic devices at the Solar Energy Research Institute in Golden, Colorado, and Dr. Fowler participated in a workshop on the toxicity of metals and their compounds used in photovoltaic devices at Brookhaven National Laboratories. Reports were made to the NIEHS selection committee.

At an NTP Steering Committee meeting in Washington Dr. Falk discussed the need and means to test for altered effects produced by interactions of chemicals on the target tissues to alert the collaborating agenices to this developing need. Ms. Jean Bernheim has looked into the literature on a series of important mechanisms of interactions.

Dr. Vouk is participating in the work of the NTP Experimental Design Group.

The staff of OHHA has become deeply involved in the preparation of the Annual Report on Carcinogens as required by law (Maguire Amendment) which is to bring to the attention of the public all important chemicals which have carcinogenic properties and which by some means or other reach the population or the environment and thus may contribute to the cancer risk for man. The report focuses on the regulatory action by a number of agencies and the way in which this action will reduce the risk of cancer in the population.

This office continued to serve towards the implementation of the Toxic Substances Control Act. Until February 1981 Dr. Piver served as the Agency's representative to the Interagency Testing Committee and continues to assist the new designee, Dr. Dorothy Canter, as the need arises. Dr. Terri Damstra continues her participation on the Interagency Toxic Substances Data Committee to design and coordinate an effective system for information retrieval of toxic substances submitted to EPA under the Toxic Substances Control Act.

Dr. Damstra also serves as NIEHS representative on the Toxicology Information Subcommittee of the HHS Committee to Coordinate Environmental and Related Programs, and on the Chemical Substances Information Network Subcommittee. She chairs the Interagency Response-to-Chemical-Concerns Committee.

Collaboration with the World Health Organization (WHO) has continued within the framework of the International Programme on Chemical Safety (IPCS). OHHA has been responsible for NIEHS' contribution to the IPCS area concerned with comprehensive evaluations of priority chemicals and with the preparation of new guidelines for drinking water quality. As WHO temporary adviser Dr. Vouk attended the second session of the IPCS Advisory Committee (Luxembourg, December 1980) and participated in a Workshop on Methods for Assessing the Effects of Chemicals on Reproductive Function (Ispra, May 1981) organized within the framework of IPCS. He also visited WHO, Geneva (May 1981) to assist in finalizing an environmental health criteria document on arsenic and its compounds.

Dr. Falk remains a member of the International Joint Commission's Committee on the Assessment of Health Effects of the Great Lakes Water Quality and is involved in the evaluation of water pollutants on the health of the population living in surrounding counties.

The Associate Director of OHHA remains active as a member of the CIIT scientific advisory panel, the Cornell University program on analysis of National Policies concerning the exposure to carcinogenic chemicals in specific countries, the Brookhaven National Laboratory Visiting Committee where he will become chairman. He also participated in the FDA's evaluation of the experiments on the carcinogenicity of nitrites and as a member of the search committee for the Director of the Division of Cancer Cause and Prevention for the NCI, and talked with faculty and students of NCSU on toxic wastes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20002-09 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Technology Forecasting and Technology Assessment

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

PI:	Warren T. Piver	Chemical Engineer	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
	Terri Damstra	Biochemist	OHHA	NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

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

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

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

The RCRA requirements for incinerator operation require destruction efficiencies of 99.99%. In order to optimize design waste load, principles of chemical reactor design are being applied to incinerators. For multi-chemical feed streams, the operations of the incinerator must initially cause the chemical to be in a gaseous or very finely divided state. In this condition, the incineration of chemical wastes is similar to a gas-phase isothermal plug-flow chemical reactor. The level of chemical destruction is a function of the

temperature and length of the isothermal section. Work is continuing to refine the bases for these conclusions and to develop correlations that can be tested in commercially available incinerators. The results of this analysis will form the basis for selection and operation of an incinerator for Institute laboratory wastes.

The selection of commercially important chemicals for mutagenicity testing is an NTP activity. Efforts so far have been towards identification of groups of chemicals that are similar to chemicals with demonstrated biological activity. At the present time, 600 individual chemicals have been recommended for mutagenicity screening in the different salmonella in vitro test systems. This work will continue because not all of the commercially important chemicals have been examined. In addition, future efforts will be towards determination of chemical reactivities of different molecular structures by application of quantum mechanical representations of these chemicals.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20003-08 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Preventive Surveillance of Environmental Chemicals for Toxic Potential

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

PI:	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Raymond E. Shapiro	Asst. Dir. Toxicol. Coord.	OD	NIEHS
	OHHA Staff		OHHA	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
OD

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE:

Compounds with a vinyl halide substructure: Research on vinyl halide-type compounds remains very active. The area is too broad to discuss briefly here. Animal in vivo and in vitro studies are expanding with regard to more complex structures.

Methanol: Some see methanol replacing ethanol as an alcohol of choice for fuel uses, either alone or mixed with gasoline or diesel fuel, in the U.S. and elsewhere. Many factors continue to make methanol subject to abuse and to accidental poisonings and to make treatment difficult. Methanol uses may better be confined to situations where it would be less available to be used for intoxication or to result in inhalation or dermal exposures.

A review of recent literature indicates that the rate of human skin absorption of methanol from the forearm is in the same range as that of benzene, xylene or carbon disulfide. When applied as a 15% solution of methanol in diesel fuel (a proposed vehicular fuel), blood concentrations were 3-4 times higher within the first hour, possibly resulting from defatting of the skin by hydrocarbons. Emissions from a methanol-diesel fuel blend appear to be more mutagenic in some of the experimental systems than that from diesel fuel alone. A paint remover spray containing 20% methanol and 80% methylene chloride resulted in increased human blood carboxyhemoglobin (due to carbon monoxide from the methylene chloride; a hazard to those with cardiac stress) and caused a death. Methanol and isopropanol equally potentiated experimental liver toxicity due to carbon tetrachloride in rats. In both rats and mice, interestingly, ethanol was less potentiating than methanol or isopropanol.

The slow excretion of methanol in humans could contribute to human toxic interactions that could occur under a variety of conditions. Infants and children appear to be as susceptible to methanol toxicity as adults. Methanol has been mistaken for both ethanol and water and poisonings have occurred in this manner. Brain CAT (computer-averaged tomography) scans in humans verify the post-mortem findings and now show clearly, in living individuals, that particular types of brain damage can occur.

It has been shown that formic acid rather than formaldehyde accounts for visual damage in humans and monkeys after methanol. Experimental therapeutic attempts have recently included folic acid and leucovorin, which probably act by stimulating the rate of metabolism of formate and, in this manner, excretion.

Paraquat: Research reports dealing with the herbicide paraquat continue to appear regularly. More rapid and sensitive assays for its concentration in serum have been developed and usual concentrations in the first hours and days after ingestion, associated with survival or death, have been cited. Either hemodialysis or hemoperfusion over activated charcoal is being recommended in more serious cases. These two methods supplement or sometimes replace some of the earlier treatments.

Sixty-eight reports of poisonings in Great Britain and 70 in France were reviewed. Liver effects have been more completely described and cerebral effects were further documented. A few reports appeared of symptomology that was suggested to be due to nebulized sprays and also an additional percutaneous fatality was reported. However, the medical records of paraquat formulators and pulmonary function tests in spraymen were said not to have indicated pulmonary symptoms. Both inhalation and intratracheal application of sufficient paraquat in experimental animals produces decrement of lung function and fibrosis.

Few structural homologs approach paraquat in its herbicidal activity. However, 2-methylparaquat (1,1',2-trimethyl-4,4'-bipyridinium) is equally as potent. Human safety and commercial application using this compound do not yet appear to be in the open literature.

With greater care in spraying marihuana with paraquat in Mexico, a smaller range and lower median contamination with paraquat was reported. However, if paraquat spraying is deemed acceptably safe and applied at many locations where marihuana is grown -- with or without a visual or odorant marker the safety of which should be considered -- higher contaminations could again occur due to the many factors that surround spraying of this widely grown and illicit crop.

Stratospheric modification: Participation on the Interagency Committee on Stratospheric Ozone Protection (ICSOP) has involved guiding the government-supported research, most particularly human health studies. A report to congress is prepared every two years. The third is scheduled for January 1, 1982. This past year, the UNEP (U.N. Environment Program) Committee on the Ozone Layer agreed that depletion of stratospheric ozone is still most likely by some chlorinated species and it may be about 1% by now, perhaps ultimately reaching 10%. The decrease has been insufficient so far to demonstrate by statistical means. An experiment this summer will assay O, ClO, O_3, and HO_2 as a balloon (on an ultralight cable) containing the instruments is repetitively reeled down and up through 20 km of stratosphere from earth. This could be done ten times in a day if necessary. Immediate resampling of a region should be possible. Presence or absence of an inverse relationship of O_3 and ClO concentrations should be the strongest data to date for or against the hypothesis.

In addition to direct human health effects of extra exposure to solar ultraviolet irradiation in the 285-320 nm range, co-exposure to particular chemical structures or other wavelengths of radiation, and effects occurring in allelic human and simpler species, are being investigated. For example, chemicals are used for sun-tanning, and for treatment of psoriasis, uremic pruritis, acne and

schistosomiasis in the presence of irradiation. Some individuals develop phototoxic or photoallergic reactions to compounds, the exposure to which is by a variety of routes. Carcinogenicity and mutagenicity are other potential or suspect effects, which in some cases are being demonstrated in the lower species and mutagenesis in human cells in culture. Clarification of the effects of the extra amount of UV and transmission of slightly shorter wavelengths, avoidance of hazardous chemicals, the development of improved testing systems, the development of safer drugs, and protection of generally more-sensitive subgroups in the population are among the goals of the research.

Aminimide compounds: Four reports between 1975 and 1977 showed antimicrobial activity against gram positive and gram negative bacteria and against two yeasts, achievable by varying chain length and other structural features around an acyl-aminimide group (an acyl amino imide; $-CO-\overset{-}{N}-\overset{+}{N}\leqslant$). Some of the compounds have more recently been shown to be active against two species of mosquito, more so for the pupal than for the larval stage. Two aminimides were tested for anti-neoplastic activity against three tumors in rats and mice. Life span could be prolonged to some extent in all systems. Cures were not seen with B16 melanoma or Sarcoma 180A in the rat. Cures occurred, however, with the Sarcoma 180A in the mouse. Cures were also seen with the T-8 Guerin tumor in rat. Preliminary toxicologic information was presented in these reports. A patent appeared exploring the use of aminimides for protecting plant materials from injury during frost and subfreezing temperatures. The literature will continue to be followed concerning these newer type compounds.

Hazards of coal gasification schemes: The literature of chemistry and biological responses to potential conversion products of gasification of coal is being followed. It is shared with others in OHHA presently more involved in this project.

Asbestos substitutes: I attended the National Workshop on Substitutes for Asbestos and continue to collect information about materials in this broad area.

Polybrominated biphenyls: I contributed to several sections of a report prepared by OHHA which will be published in ENVIRONMENTAL HEALTH PERSPECTIVES.

Card file of chemical and physical agents considered in NIEHS intramural research: The card file begun and described previously has been continued. It has been used on request to identify those individuals at NIEHS involved with particular chemical and physical agents.

Other NIEHS and outside activities: Documents are reviewed for their health hazard aspects as requested and requests for information are answered.

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

PUBLICATIONS

Posner, H. S.: Biochemistry by Armstrong, F. B. and Bennett, T. P. (Book Review). The Public Health Lab. 38: 329, 1980.

Posner, H. S.: Pesticides. In Academic American Encylopedia. Princeton, Aretê Publ. Co., Inc. 1980, 15, pp. 196-199.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20004-07 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Surveillance of Potential Environmental Health Hazards

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

PI:	Terri Damstra	Biochemist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Warren T. Piver	Chemical Engineer	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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 (200 words or less - underline keywords)

The objectives of this long-term project are: (1) to compile an index of associations between organ system diseases and symptoms in humans and exposure to environmental and occupational chemicals; and (2) to assess the availability, validity, and utility of using neurological and behavioral tests as early indicators of potential toxicity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparation of an open-ended data file, which is continually updated via constant literature surveillance along with computer access to various bibliographic and numerical data banks. Membership on committees; attendance at meetings; reviews of documents and manuscripts; evaluation of computerized data files; preparation of reports and monographs; and consultation with scientists from other government agencies, industry, and academia.

MAJOR FINDINGS AND PROPOSED COURSE:

The diseases, syndromes, and symptoms associated with several organ systems have been identified and indexed. A preliminary list of associations between exposure to environmental and occupational chemicals and human nervous system, respiratory system, cardiovascular system, and renal system diseases has been compiled. Information relating symptoms and diseases to chemicals will continue to be collected.

In some instances cause/effect relationships are clearly established, whereas the evidence for other associations is limited to a few anecdotal case reports. Many symptoms are nonspecific and can be attributed to numerous factors. It is therefore difficult to correlate such symptoms with chemical causes unless some preliminary warning of the possible hazard has been given. Nevertheless, these symptoms may be the first sign of intoxication with environmental chemicals.

There is at present a lack of rational criteria for selecting suitable experimental procedures to determine toxic neurobehavioral effects in both animal test systems and human populations. Methods for monitoring neurobehavioral effects in exposed humans and in animal test systems will continue to be evaluated. Particular emphasis will be placed on test systems used to detect neurobehavioral toxicity in developing organisms as a result of *in utero* exposure to toxic agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A compilation of associations between target organ toxicity and exposures to chemicals will aid the Institute in making a proper assessment of potential health hazards and in identifying the research programs necessary before such assessments can be made. An index of toxicological symptoms and diseases will also assist in the differential diagnosis of diseases considered likely to be of toxicological origin.

PUBLICATIONS

Damstra, T. and Bondy, S. C.: The current status and the future of biochemical assays for assessing neurotoxicity. In Spencer, P. and Schaumberg, H. (Eds.): Experimental and Clinical Neurotoxicology. Baltimore, Maryland, Williams and Wilkins Company. pp. 820-833, 1980.

Rogan, W., Bagniewski, A., and Damstra, T.: Pollutants in breast milk. N. Engl. J. Med. 302: 1450-1453, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20007-04 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

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

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

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

COOPERATING UNITS (if any)
Institute-wide

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to identify and evaluate real and potential health hazards in the environment with emphasis on chemicals both as toxicants and as carcinogens.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

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

2. The Principal Investigator prepared a series of reviews that included "Enzyme Induction by PBBs," "Chemical Interaction of PBBs with other Chemicals and Drugs," and "Absorption, Metabolism Distribution and Excretion of PBBs." These papers will be published in EHP as part of a review titled "The Toxicity of PBBs (BP-6 or FF-1) in Domestic and Laboratory Animals."

3. The Principal Investigator prepared a review of chemical interactions in carcinogenesis for the Subcommittee for the NTP Chemical Nomination and Selection Process Committee.

4. A review of Caffeine as an Environmental Agent has been initiated for publication. The review will summarize and interpret the available data on the biological effects of caffeine with the dual objectives of reaching a conclusion on its safety and compiling recommendations for further experimental efforts. Most of the pertinent references have been collected and information on work on caffeine in process is being compiled.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20008-04 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

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

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Health Hazard Assessment

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Special histologic techniques, light and electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Carcinogenesis Study. (a) Work continues in an attempt to complete for publication the characterization of the lesions induced in developing opossums with ethylnitrosourea (100 mg/kg). (b) A paper titled "Chemical Induction of a Neurogliogenic Tumor: An Accurate Model for the Human Ganglioglioma" was presented at the annual meeting of the American Association of Pathologists in San Diego, California, and a paper entitled "Chemical Induction of Embryonal Renal Tumors: An Accurate Model for Pediatric Renal Neoplasia" was given at the annual meeting of the American Association for Cancer Research in Anaheim, California. (c) A review titled "The Marsupial as a Biomedical Model" has been completed and submitted for publication. (d) Collaborative Studies and Consultation. The principle investigator is collaborating in several studies utilizing the opossum, being conducted or planned at other institutions.

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

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

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

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

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20009-03 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Identification of Potential Environmental Health Hazards

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

PI: Jean Bernheim Microbiologist OHHA NIEHS

COOPERATING UNITS (if any)
Institute-wide

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4		0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The aim is to identify and evaluate potential health hazards in the environment from exposure to chemicals by an in-depth search of the literature. The mechanism of action of the toxicant is the ultimate goal of this endeavor.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE:

Love Canal: A tabularization of the available toxicity data with references was compiled for the approximately 300 chemicals which have been identified at Love Canal. This was included in the report of the DHEW CCERP Subcommittee on the Potential Health Effects of Toxic Chemical Dumps that was released at the Eckhardt-Moffett hearing on May 22, 1980. This file is being continuously expanded should information on a specific chemical with known human exposure be needed for toxicological evaluation.

Chemical/Chemical Interactions: This is a long-term project which has been initiated because of the multiple nature of most chemical exposures of the human population. An open-ended file has been started to collect specific types of interaction and of the most appropriate experimental methods available for testing.

DNA Repair Enzyme Induction: An adaptive response -- an "error free" repair pathway -- is induced in bacteria during growth in low concentrations of various alkylating mutagens rendering the bacteria more resistant to the toxic and mutagenic effects of these chemicals. This repair induction has also been demonstrated in rat liver DNA, by measuring the increased excision of O^6-methylguanine, after chronic administration of N-nitrosamines. This may be a significant factor in organ-specific carcinogenicity as related to dose of the chemical.

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

Somatic Cell Hybrids: Studies of malignancy in cell hybrids (intraspecies and interspecies) formed between tumorigenic and nontumorigenic cell has been hampered by an inability to dissociate controls acting at the level of the chromosome from those having an extranuclear or epigenetic basis. Construction of somatic cell hybrids has revealed either suppression or expression of the malignant phenotype, depending on the cell types used for fusions. Interspecies hybrids may show "chromosome segregation" frequently involving preferential elimination of chromosomes of one species with selective retention of chromosomes of the other species. This provides a method of associating genetic function with specific chromosomes in eukaryotic cells, although the activity of some extrachromosomal element (i.e. centrosome) may also be involved.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information available in the literature often times can give us clues on potential hazards that may be anticipated on exposure to new chemicals which are bearing close relationship to other better known chemicals. The mistake of making too sweeping generalizations as has often been done in the past has tended to discredit this structure/activity correlation, but when done with proper care and limitation it is a very good tool for hazard assessment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20010-01 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Identification and mechanism of action of DNA repair inhibitors

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

PI: Jean Bernheim Microbiologist OHHA NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6		0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

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

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20011-01 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

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

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

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

* WHO Interregional Research Unit located at NIEHS

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

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this long-term project, carried out within the WHO/UNEP/ILO International Programme on Chemical Safety (IPCS) is to prepare balanced evaluations of biolgoical effects and health hazard assessments of selected chemicals, including evaluations of toxicological methods on which published data are based.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, reviewing and selecting relevant publications, preparing of data files which are continuously updated, consultation with national focal points and appropriate individual scientists; preparation of draft environmental health criteria and similar documents which are then reviewed, and revised by an international group of experts, convened by the Central Unit, IPCS, Geneva, or by NIEHS on its behalf; scientific editing of documents approved by international groups of experts.

MAJOR FINDINGS AND PROPOSED COURSE:

2,6-Toluene diamine: This chemical is a byproduct of the synthesis of 2,4-toluene diamine. Mixtures of 2,6-toluene diamine and 2,4-toluene diamine are used in the synthesis of toluene diisocyanate which is the predominant isocyanate applied in the production of polyurethane foams and elastomers. 2,6-Toluene diamine is also applied as an intermediate in the production of dyes for furs and textiles. Since 2,4- and 2,6-isomers are used mostly as mixtures, they have to be evaluated together. 2,4-diaminotoluene is carcinogenic in rats but the 2,6-isomer was not found carcinogenic in rats or in mice. A preliminary draft of the evaluation document has been prepared.

Phthalic acid esters: These plasticisers are produced in large volumes, and seem to be rather widely-distributed in the environment. A recent NTP bioassay report indicated that di(2-ethyl hexyl) phthalate (DEHP) is carcinogenic in Fischer 344 and B6C3F$_1$ mice. This has received national and international interest in phthalic acid esters as environmental pollutants. The preparation of an environmental health criteria document is in progress.

Principles and methods for toxicity evaluation of chemicals: A valid international evaluation and health hazard assessment of chemicals depends on the comparability of results obtained in different laboratories nationwide and worldwide, and this in turn depends on the understanding of principles, approaches, and methods used for toxicity testing. With this in mind, WHO published in 1978 the first volume of a monograph on principles and methods for evaluating the toxicity of chemicals (Environmental Health Criteria 6, UNEP/WHO, Geneva). The manuscript of the second volume of this monograph is being edited and updated. This volume will discuss tests for: organ systems functions; effects on reproduction, embryo- and fetotoxicity; neurological and behavioral effects; effects on skin and the eye; cumulation and adaptation; and modifying factors.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20012-01 OHHA

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Evaluations of Toxicological Mechanisms and Health Hazard Assessment of Selected Chemicals

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

PI: Janet Guthrie Microbiologist OHHA NIEHS

COOPERATING UNITS (if any)

WHO Interregional Research Unit located at NIEHS

LAB/BRANCH
None

SECTION
None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5		.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Three main focuses of this work are: (1) Developing a compendium of current research and results on various topics of toxicological significance; (2) Developing a document designed to provide balanced evaluation of biological effects and health hazard assessments of selected chemicals; and (3) Assisting in the development of a document on criteria of methods to be used in the toxicological evaluation of various compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: All of the above projects involve extensive literature searches for pertinent publications. Additionally, for some projects communications are established with major researchers in the field to learn of their current, as-yet-unpublished work.

MAJOR FINDINGS AND PROPOSED COURSE:

I. Data were compiled on the following research topics:

A. The emergence of prostaglandin synthetase as a system able to co-oxygenate various xenobiotics to active mutagens/carcinogens.

B. The greater significance of nuclear AHH, as compared to cytoplasmic AHH, in the activation of xenobiotics and their subsequent interaction with DNA, an event of prime importance to mutagenesis and carcinogenesis.

C. Evidence, in some compounds, of a dose-response curve of a non-linear nature.

D. Elucidation of the receptor mechanism responsible for the induction of AHH (the so-called "TCDD receptor").

The findings on these different topics have been compiled into a brief report form which has been shared with interested scientists both within and outside of the agency.

II. Data were compiled on the following selected chemicals:

A. 4,4'-oxydianiline -- this chemical is used in the manufacture of high temperature resistant metal adhesives, molding and machine parts, and insulators. It was found to be both mutagenic and carcinogenic, but toxicological effects of a more subtle nature (e.g., effects on the immune system and reproduction) have not been studied.

B. Phthalic acid esters -- These plasticisors are produced in large volumes, and seem to be rather widely-distributed in the environment. A recent NTP bioassay report indicated that di(2-ethyl hexyl) phthalate (DEHP) is carcinogenic in Fischer 344 and B6C3F$_1$ mice. This has received national and international interest in phthalic acid esters as environmental pollutants. The preparation of draft environmental health criteria documents is in progress.

III. Reports that have been suggested for publication are presently undergoing scientific editing prior to being sent to the authors for updating. The topics of these reports are methods for the evaluation of toxicological effects on skin and eye and evaluation of the accumulation and adaptation of toxicants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

I. These individual reports represent the most current thinking on various topics of toxicological significance. These reports have been used by both scientists in and outside of the agency and have been of particular value because of inclusion of as-yet-unpublished data.

II. The results of literature searches on 4,4'-oxydianiline were of value in spotlighting the paucity of information both on the toxicological effects of very low doses and of very subtle (i.e., not detected by gross examination) disturbances to the biological organism.

III. This on-going work is significant because it will provide, at an international level, standards for toxicological evaluation. Its adoption by the research community will greatly enhance the possibility of comparing the results obtained among different laboratories.

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

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

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

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

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $104,680

PROJECT DESCRIPTION

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

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

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

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

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

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

Distribution and the beginning stages of chemical form identification for nickel and cadmium in soybean plants have also been completed. At senescence, >70% of Ni present in the stem was remobilized to the seeds. Nickel accumulated in seeds was primarily associated with the cotyledons. In the cotyledons, 80% was associated with the soluble whey fraction and 70% of this fraction was composed of Ni-containing components with MW <10,000. The behavior of Cd^{+2} was much different from Ni^{+2}. Cadmium was strongly retained by the roots after absorption. Of the tissues examined, cadmium was found mostly in the soluble fraction with >80% being associated with components >10,000 MW. The cadmium that does reach the seeds was associated with the soy whey of cotyledons. Studies are continuing to determine the amino acids that comprise the metal complexes.

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

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, legislative support and related activities. The most significant of these are summarized below. As in last year's reports these are summarized in two ways:

1. Areas of substantive program activity.

2. Areas of functional activity.

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

Substantive Program Activities

Toxic Chemical Waste Dumps: (Ms. Hudson)

During the time it had the "Superfund" legislation under consideration the Subcommittee on Environmental Pollution of the Senate Committee on Environment and Public Works asked the Surgeon General to provide his assessment of the threat to the public health posed by toxic chemicals in the United States. OPPE drafted the Surgeon General's report. In final form, this report discussed the Public Health Service's (PHS) general views regarding the threat to ongoing DHHS activities that should help answer the questions relating to toxic chemicals in the years ahead. (While this report was submitted at the end of FY 1980 it was not discussed in the FY 1980 Annual Report.) This paper was widely quoted in the newspapers and in the scientific and technical press at the time it was released and continued to be referred to throughout 1981.

DHHS/IOM Cost of Illness Study: (Mr. Kingman)

The Health Services, Research, Health Statistics, and Health Care Technology Act of 1978 (PL 95-623) directed that DHHS and the National Academy of Sciences/Institute of Medicine (NAS/IOM) jointly conduct a study to determine that portion of the cost of illness which is related to environmental factors. As a result of that legislation the Department contracted with the IOM for the purpose of developing a plan for the required study. The IOM committee, composed of individuals representing a wide array of disciplines, met throughout FY 1980 and into early FY 1981.

Because of the public policy importance of the proposed study the OPPE had a major interest in this activity and, as a result, was asked to represent the Director at meetings of the committee.

When the IOM submitted its report in late January, 1981 the DHHS Committee to Coordinate Environmental and Related Programs (CCERP) was asked to review it and make recommendations to the Secretary regarding the appropriate response. The OPPE provided staff support to the Chairman of CCERP in carrying out this effort. The drafting of the response was carried out by OPPE, as well as clearance with the numerous PHS agencies which were involved.

In summary the group recommended that the Secretary defer implementation of the study on the basis that the scientific data base is not adequate to permit undertaking the study at this time, and that fiscal stringencies militate against undertaking this venture as a pilot activity.

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

In late Winter, 1981 the Subcommittee on Investigations and Oversight of the House Committee on Science and Technology announced its plans to hold oversight hearings on the NTP. The Subcommittee viewed NTP as an important experiment in interagency collaboration, and as it has been in existence for almost three years believed this is an appropriate time to evaluate it.

OPPE had the primary Institute responsibility for preparing for the hearing, and for serving as principal Institute liaison with Subcommittee staff.

As part of its responsibility for this hearing the OPPE prepared an exhaustive paper, detailing the history of NTP's establishment, difficulties inherent in the NTP model, NTP accomplishments since its founding, the Office of the Assistant Secretary for Management and Budget (MB) evaluation of the program, and future directions for NTP. To develop this paper, interviews were held with key NTP staff members.

During the year OPPE provided staff support for other NTP-related activities. OPPE developed the NTP response to the Office of Science and Technology Policy (OSTP) concerning programs that might be enhanced or begun to meet the President's economic revitalization objectives. OPPE developed a background paper on NTP for the Department's option paper regarding coordination between the DHHS and the Environmental Protection Agency (EPA), as well as the response to public comments on the NTP research initiative, which had been developed for inclusion in the Report of the DHHS Steering Committee for Development of a Health Research Strategy. OPPE staff also reviewed the Bureau of Foods 1980 Research Plan to determine areas in which the NTP could offer assistance to the Food and Drug Administration (FDA); coordinated preparation of material on NTP for the National Science Foundation (NSF) Five-Year Outlook for Science and Technology and the Annual Science and Technology Report; and provided input for comments on the MB evaluation of the NTP.

With regard to the MB evaluation of the NTP, the study team reported it found indications of initial success by the NTP in moving toward each of its goals, and recommended that NTP's experimental designation be removed and that NTP be established as the permanent mechanism for coordinating the Department's toxicological research. The study team expressed its belief that the NTP offers the highest probability for success because of the experience it has gained and the relationships it has developed over the two years of its existence. At this time the Secretary is considering the MB recommendations. On July 14, 1981 the Secretary approved the NIH to transfer the NCI Carcinogenesis Program to the NIEHS, thereby consolidating the NIH contributions to NTP within the NIEHS.

Health Promotion and Disease Prevention: (Ms. Hudson, Mr. Kingman)

Throughout the past year OPPE has been called upon to participate in and contribute to the Assistant Secretary for Health (ASH) effort to develop a PHS-wide program in health promotion and disease prevention. This effort has required

continuing input for and review of ASH-developed documents. For example, OPPE provided information on the NIEHS primary prevention efforts and reviewed the 1990 measurable prevention objectives. OPPE also coordinated preparation of material on NIEHS prevention projects for use by the Director at a briefing for the new DHHS Secretary, who pledged to continue emphasis on prevention.

More recently OPPE has been called upon to provide staff support to the Director--in his role as Chairman of the DHHS Committee to Coordinate Environmental and Related Programs (CCERP)--and worked with staff of the member agencies to develop an implementation plan for the toxic agent control objectives outlined in the PHS report Promoting Health/Preventing Disease: Objectives for the Nation. Building on this effort, OPPE is engaged in leading the PHS-wide effort to develop compendiums of ongoing DHHS, other Federal agency, and private and public activities related to the toxic agent control objective for use by ASH in planning PHS activities.

Program Planning Activities: (Ms. Hoffman)

The OPPE formalized program planning activities are driven by the requirement for the development of an annual three-year plan. While this activity has utility for the NIEHS, its impact on the acquisition of additional resources has been marginal. The formal budget process continues to be the principal means by which those fundamental resource issues are resolved. (For this reason close collaboration between the OPPE and the Budget Staff has been essential.)

In addition, the OPPE has been involved in a variety of long-term, continuing and one-time planning activities. Among these are:

1. Efforts to redesign the Institute's budget activity structure, which was carried out at the request of the Director, NIEHS.

2. Preparation of the Institute's 1983 Research Plan.

3. Preparation for the Director, NIEHS's program planning review session with the Director, NIH.

4. Service as member of the NIH-wide working group to review the NIH planning process.

Program Evaluation Activities: (Ms. Hudson)

A companion to the formal planning process is the formal program evaluation process, which is part of a Department-wide activity.

The evaluation planning cycle for FY 1982 got underway during the Winter of 1981 when OPPE developed the NIEHS FY 1982 Evaluation Plan. Subsequently NIH, ASH, and the Office of the Secretary (OS) program evaluation staff reviewed NIEHS' plans for the upcoming year and the progress of NIEHS' evaluation projects.

Currently, NIEHS is in the process of completing three evaluation projects, two of which are being funded through the Department's set-aside evaluation program. The one non-set-aside project concerns the Program Planning Work Group, which was asked to review the recommendations contained in the Report of the Second Task Force for Research Planning in Environmental Health Science, and to make

recommendations regarding research areas of particular relevance and importance to the NIEHS. The second evaluation project focuses exclusively on the NIEHS Environmental Toxicology Training Program and is designed to evaluate the effectiveness of that training program and to determine the supply/demand situation for toxicologists. The third evaluation project was undertaken during FY 1979 - 1981 at the request of the Office of the Secretary, DHHS. In order that the Secretary could advise the Secretary of State, as required by law, NIEHS was asked to provide the necessary exposure data from the smoking of paraquat-contaminated marijuana to assist in evaluating any potential health hazards of smoking such contaminated marijuana in the United States.

During the coming year, NIEHS program managers and staff will continue to rely upon the NIEHS evaluation process--which depends to a great extent on the NIEHS formally constituted non-governmental review groups, the National Advisory Environmental Health Sciences Council (NAEHSC), the NIEHS Board of Scientific Counselors and the NTP Board of Scientific Counselors--for assessing the effectiveness, responsiveness, and contribution of Institute programs. In looking to the years ahead, OPPE plans to better integrate the Institute's program planning and evaluation activities to improve their utility.

Legislative Analysis Activities: (Ms. Hudson, Ms. Stopinski)

The development of effective program plans in environmental health requires a knowledge of and appreciation for the relationships between research and regulation. The development of public policy through the legislative process is at the center of those relationships. Thus, the OPPE has found it necessary to devote considerable energy to the ongoing tracking and analysis of much legislation in this area. This activity is designed to keep the Institute staff informed of legislative developments in Congress and to ensure that NIEHS has the opportunity to provide input into legislative and executive matters of interest and concern to it.

OPPE activities in relation to legislative information and analysis in 1981 were wide-ranging and diverse. They ranged from developing and maintaining a legislative library dealing with topics of immediate or potential interest to the Institute and key staff (such as "Superfund," toxic waste dumps, dioxin, and air pollution), to tracking bills of interest through the legislative process, to researching the background of proposed and enacted legislation. OPPE prepared memos of alert for the NIEHS Director, as well as NIH staff, regarding the implications and requirements of proposed legislation; developed recommendations for the NIEHS Director, DHHS and PHS officials, and Congress on proposed and enacted legislation; accompanied the NIEHS Director and other key staff to hearings; edited and prepared inserts for hearing records; developed statements, briefing books, and background material for testimony by the Institute Director, as well as NIH and DHHS officials; and developed briefing information for Congressional leaders and staff. In addition an extensive analysis of the issues related to air pollution legislation was prepared as background material for the Director.

To assist in keeping the Director's staff abreast of the latest legislative issues, a list of upcoming hearings is prepared and sent to the OD staff weekly when Congress is in session. OPPE also maintains working relationships with key legislative information and support services such as the Congressional Research Service.

Administrative Considerations

As pointed out last year there are a variety of administrative considerations which impact on the work of OPPE. As before, foremost among them is the current severe stricture in personnel staffing. This is particularly difficult because a relatively small amount of its workload is determined by OPPE. In order to be effective the OPPE requires a staff which is interdisciplinary and provides the Institute with expertise that it does not currently possess. Among these needed disciplines are law, economics, and political science. For a group which needs to be collegial in its approach to its tasks, the results are limited by the range of disciplines represented. The temporary assignment of Dr. Marshall Plotka (from October 1980 to June 1981) provided a broadening of OPPE staff competance which permitted OPPE to consider a variety of different policy issues. His departure to begin his residency left an important gap in OPPE. Thus, strictures in staffing not only limit the amount that OPPE can accomplish, but also restrict the range of activities it can effectively encompass. It is hoped that these limitations will be eased at least a little in the coming year.

In terms of personnel management, appropriate grades for OPPE staff are extremely difficult to establish because of the absence of appropriate position classification standards for program analysts. Recognizing this issue, in 1980 the NIH Institute Planning Officers began a study of the NIH program analysis positions in an effort to propose an appropriate standard to the Office of Personnel Management. This project was delayed during 1981 and it is unlikely that this issue will be satisfactorily resolved before 1983. In the interim, classification of these positions will continue to be more art than science.

Finally, it must be recognized that for an office like OPPE the research, thought, and writing are to no avail if the product does not appear on the page. Thus, the development of a strong clerical support staff has been critical to the effective functioning of OPPE. Inability to replace one of the clerical staff who left the Institute, has placed the remaining staff in a difficult workload situation. It is clear that OPPE must soon move into word processing to reduce the lag between preparation and issuance of OPPE products.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement - FY1981

During FY1981 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 Meeting of the Panels, held in Kyoto, Japan, September 28, 1981 reviewed the information presented at the Third International Conference on Environmental Mutagens held in Tokyo September 21-24, 1981, and Kyoto September 25-27, 1981, and served as a planning session for future collaborative programs and workshops of the Joint Panels.

US-USSR

In continued exploration of the significance of a 1000-fold higher frequency of electrophoretic variants among congenitally malformed children than in normal children in the USSR (as reported by Dubinin et. al), the OADG has arranged for the visit of the Soviet scientists Drs. Y. Paschin and Y. V. Altukov in the laboratory of Dr. J. V. Neel at the University of Michigan. It is hoped that studies of blood samples from Russian children in Dr. Neel's laboratory may resolve the meaning of these unexpected findings in the Soviet Union. In addition, in cooperation with the National Academy of Sciences (US), the OADG coordinated the visit of Dr. Galina Zasukhina, Academy of Science (USSR), to the Research Triangle Park during the period November 24 - December 1, 1980.

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, May 2-9, 1981. During this period as Vice-Chairman the ADG participated in a review of ICPEMC activities including an evaluation of ongoing work of the five Committees. This review consisted mainly of an evaluation of individual working papers prepared for inclusion in the final committee reports.

National Programs

EPA Gene-Tox Program

At an Open Meeting held in Washington, DC December 3-5, 1980, the final reports of the 25 workgroups of experts in the EPA Gene-Tox Program were presented. The ADG, representing the Steering Committee, initiated the second phase of the program and explained the functions and goals of the Assessment Panels which will evaluate the utility of the various test systems, cross index the data presented, and arrive at recommendations of appropriate batteries of tests for mass screening. Additional meetings of the Coordinating Committee (former Steering Committee) have been held to review the assay system reports, and to begin the data collation required to achieve the final goals of this program during FY82.

NIEHS Sponsored Conferences and Workshop

As a member of the Steering Committee, the ADG abetted in the planning and coordination of the Conference on the Evaluation of Human Populations Exposed to Potential Mutagenic and Reproductive Hazards, which was held under the auspices of the March of Dimes in Washington, DC on January 26-27, 1981. The Conference was cosponsored by the Centers for Disease Control, the Environmental Protection Agency, the March of Dimes, and the National Institute of Environmental Health Sciences. The proceedings of the Conference are in the process of being published.

The OADG coordinated cosponsorship by NIEHS of a Conference on Genotoxic Effects of Airborne Agents which was held at Brookhaven National Laboratory, Long Island, NY on February 9-11, 1981. The Conference was also sponsored by EPA and the Department of Energy.

A Workshop on Statistical Analysis of In vitro Tests for Mutagenicity was organized by the OADG and held at Chapel Hill, NC, April 21-23, 1981. During the course of this Workshop, an evaluation was made of the statistical methodology being used to evaluate data from (1) the Salmonella histidine reversion assay, (2) the Chinese hamster ovary HGPRT cell culture assay in mammalian cells and (3) the mouse lymphoma L5178Y thymidine kinase cell culture assay. A draft report of the proceedings of this workshop is being prepared for publication.

A Symposium on Molecular and Cellular Mechanisms of Mutagenesis held at Gatlinburg, TN, April 6-9, 1981 was cosponsored by NIEHS through the Office of the Associate Director for Genetics. The ADG and several members of the NIEHS scientific staff were key participants in the Symposium, the proceedings of which are in the process of being published.

Committees

Subcommittee on Environmental Mutagenesis

The ADG continues to chair the DHHS/CCERP Subcommittee on Environmental Mutagenesis. Due to budgetary and travel restrictions the number of

meetings has been curtailed to approximately 5 meetings for this FY. Nevertheless, current issues of importance to government agencies concerned with genetic toxicology continue to be addressed by speakers in the forefront of their fields. Some of the topics for FY81 were the purpose and functioning of the Environmental Mutagen Information Center; reviews of the FDA's mutagenicity testing of chemicals on the GRAS-list, proposed mutagenicity testing contracts in the National Toxicology Program and the Environmental Mutagen Test Development Program (EMTDP). The activities of the Association of Official Analytical Chemists in developing standardized protocols for mutagenicity tests was also discussed.

Federal Formaldehyde Panel

The ADG participated as a member of the Federal Formaldehyde Panel organized by the U. S. Consumer Product Safety Commission. The report prepared by this Panel was considered invaluable by the Commission and other Federal agencies attempting to determine the appropriate public policy response to the health risk posed by formaldehyde.

Collaborative Studies

WHO - International Program for Chemical Safety

The ADG has been named Chairman of the Steering Committee for a major new collaborative study -- the International Program for Chemical Safety -- sponsored by the World Health Organization (WHO), the United Nations Environmental Program (UNEP) and the International Labor Organization (ILO). It is anticipated that laboratories in the member nations will participate in the study. A meeting of an ad hoc working group chaired by the ADG was held in Geneva, Switzerland April 30 - May 1, 1981 to identify test compounds, potential assay systems and investigators from member countries and to plan the coordination of the different phases of this study: (1) evaluation of *in vitro* short-term tests for mutagenesis and carcinogenesis, (2) evaluation of short-term *in vivo* assays for mutagenicity and (3) the development of standardized protocols for the performance of short-term tests for mutagenicity during this collaborative study.

WHO - Genetic Monitoring for Environmental Effects

In addition, the ADG participated in the WHO Consultation on Genetic Monitoring for Environmental Effects, Ottawa, Canada, October 17, 1980. The purpose of the consultation was to discuss how to evaluate effects of exposure of individuals in the human population to mutagens. The major focus was on establishing guidelines whereby data, gathered on an international scale, could be collected for evaluation of human exposure to environmental mutagens.

International Program for the Evaluation of Short-term Tests for Carcinogenicity

The full report on the International Program for the Evaluation of Short-term Tests for Carcinogenicity was published in July 1981. The book

edited by the ADG and Dr. John Ashby, ICI, United Kingdom, presents all of the data collected in this study with the 30 different assays in addition to chapters containing overall evaluations and summaries.

Collaborative Research Programs

Illinois State University

The analysis of the mutagenic activity of various classes of chemical carcinogens as well as base-line chemical mutagens has continued in wild-type and excision-repair deficient two-component heterokaryons of Neurospora crassa. This contract work, which is being brought to completion at the end of FY81, is being performed at Illinois State University, Normal, Illinois. Studies on chemical carcinogens are designed to determine whether this class of chemicals produce some characteristic type of genetic change. In addition, the action of various base-line chemical mutagens is being compared in two-component heterokaryons homozygous for uvs-2 (excision-repair deficient) and those heterozygous for uvs-2 with the normal wild-type strain ($uvs\text{-}2^{+}$). These experiments provide data useful for risk-estimation since one can mimic experimentally the type of genetic diversity that is expected in our heterogeneous human population.

During this contract year a total of 14 chemicals have been tested for mutagenicity using the ad-3 test system.

Public Lectures

1. International Mutagenesis Symposium, Ottawa, Canada, October 14-16, 1980, "Some Aspects of Chemical Mutagenesis and Human Population Monitoring -- An Overview".

2. Association of Official Analytical Chemists 94th Annual Meeting, Washington, DC, October 20-23, 1980, "In Vitro Collaborative Studies".

3. EPA Gene-Tox Program, Washington, DC, December 3-5, 1980, "Review of Assessment Panel Activities".

4. American Association for Advancement of Science, Toronto, Canada, January 3-8, 1981, "Short-term Biological Tests for Identifying Mutagenic/ Carcinogenic Substances".

5. Symposium on "Short-term Tests for Carcinogenesis: Quo Vadis?", Montpellier, France, February 5-6, 1981 (Decision making Tests for Carcinogenic Evaluation) "Assessment of the International Program for the Evaluation of Short-term Tests for Carcinogenicity".

6. Symposium on Trends in Bioassay Methodology: in vivo, in vitro and Mathematical Approaches, Washington, DC, February 18-20, 1981, "Summary of the International Program for the Evaluation of Short-term Tests for Carcinogenicity".

7. 12th Annual Environmental Mutagen Society Meeting, San Diego, California, March 5-8, 1981, "Effects of Heterokaryotic State of the uvs-2 Allele in Neurospora crassa on Mitomycin C - induced killing and Mutation".

8. Symposium on Molecular and Cellular Mechanisms of Mutagenesis, Gatlinburg, TN, April 5-9, 1981, "Mutagenic Mechanisms in Lower Eukaryotes".

9. Gordon Conference - Genetic Toxicology Bioassays, New London, NH, June 29 - July 3, 1981, "Results and Problems on an International Multilaboratory Study".

10. 3rd International Conference on Environmental Mutagens, Tokyo, Japan, September 21-27, 1981, "International Program for Chemical Safety: Evaluation of Short-term Tests for Mutagenicity".

INTERAGENCY PROGRAMS

OFFICE OF ASSOCIATE DIRECTOR FOR INTERAGENCY PROGRAMS
Summary Statement

The Associate Director for Interagency Programs is responsible for the following program areas:

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 heart disease, cancer, arthritis, influenza and acute respiratory diseases, and health problems associated with environmental pollution. The Director, NIEHS, is U.S. Coordinator for the environmental health activities under the Health Agreement.

1981 was the ninth year of formal collaboration in environmental health research between the US and USSR. The first year was concerned largely with establishing working relationships and agreeing on areas of joint study. Cooperative research efforts were initiated in the second year of the agreement and involved exchange visits between scientists of both sides. The research results developed during the second and third years of collaboration were presented by American and Soviet scientists at a 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. The results of these symposia have been published in both countries. Scientific results from cooperative research during 1978 and 1979 were presented at the third joint symposium in Suzdal, USSR, in October, 1979.

Collaborative research efforts are currently divided into three problem areas aimed at (1) development of approaches for the quantitative evaluation and prediction of the biological effects of environmental chemical agents; (2) study of the long-term biological effects of environmental chemical agents; and (3) study of the long-term biological effects of physical factors in the environment.

Over 50 scientific papers have been published by American and Soviet scientists on the results of environmental health research conducted to date under this agreement. In addition, a Russian-English Glossary of Environmental Health Terminology was published in both countries to assist the communications between scientists of both sides.

The *Agreement on Cooperation in the Field of Environmental Protection* between the US and USSR addresses some of the most significant aspects of problems in the environment and is focused on the area of biological and genetic effects of pollution. This agreement is under the general direction of the Administrator, EPA. The Director, NIEHS, serves as DHHS representative to the agreement and as Co-Chairman of the working group for the section on Biological and Genetic Effects of Pollution. Joint work in this area, which involves scientists from NIEHS, EPA, and various universities on the American side, is concerned with the mutagenicity of environmental contaminants, the toxicity of oil shale products and by-products, neuroendocrine toxicity and behavioral effects, the effects of pollution in the marine environment, structure-activity relationships, and the

biological effects of ultraviolet light.

US-China Cooperation

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

Energy-Related Research

NIEHS is involved in a variety of interagency activities to elucidate the potential adverse health impacts of energy technologies as part of the high priority accorded by the Federal Government to solving the nation's energy problems.

During 1974 an OMB/CEQ task force met to identify needed research on the potential adverse health and environmental problems associated with energy use. As part of the report of the Interagency Working Group on Health and Environmental Effects of Energy Use published in 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. The energy-related research being pursued by NIEHS focuses on mutagenic effects; teratogenic and reproductive effects; behavioral and neurotoxic effects; inhalation toxicity and pulmonary effects; subcellular, cellular and organ toxicity; pharmacologic effects; and the determination of immediate and long-term effects of critical pollutants on selected ecosystems and organisms in the marine environment. From 1975-1980, NIEHS initiated a number of new projects focused on problems in these areas.

In 1977 the President directed DHHS, EPA, and DOE to establish a joint program to identify the health and environmental effects of emerging energy technologies. The three agencies jointly sponsored a series of scientific workshops to obtain an up-to-date identification of the health and environmental problems associated with various energy technologies and the research needs required to address those problems. In 1977 the President's Message on Energy announced the intention to appoint a special committee to study the health and environmental effects of increased coal production and use. The Director, NIEHS, was appointed Chairman of the Committee which reported its findings and recommendations to the President at the end of the year. The report of the committee and the eleven papers presented at the meetings of this Committee were published in Environmental Health Perspectives.

Interagency Coordination

During 1976 and 1977, a report on Federal Agency Support for Environmental Health Research was prepared at the request of the Senate Appropriations Committee. This report was updated in 1978, 1979, and 1980 at the request of the Director, Office of Science and Technology Policy, Executive Office of the President. The report summarizes the environmental health research responsibilities, functions, and coordination efforts of the Department of Health and Human Services, Department of Energy, Environmental Protection Agency, Department of Agriculture, Department of Commerce, Department of Interior, Department of Defense, National Science Foundation, Nuclear Regulatory Commission, National Aeronautics and Space Administration, Department of Housing and Urban Development, Department of Transportation, and Veterans Administration. These agencies budgeted approximately $728 million for environmental health research during FY 1981.

As a service to the National Toxicology Program, the Office of Interagency Programs annually prepares a Review of Current DHHS Research Related to Toxicology. In 1981 the review was expanded to include the Environmental Protection Agency and the Department of Energy. This review is required as a part of the annual plan for the National Toxicology Program. It surveys agencies of the Public Health Service, Environmental Protection Agency, and Department of Energy for information on programs in basic toxicology research, toxicology testing, and toxicology method development. Twenty-one agencies reported funding for toxicology-related research amounting to approximately $314 million in FY 1981. The review also identifies the chemical compounds under test for a variety of biological endpoints and toxicology test methods currently under development.

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

Facilities: The Office of Facilities Engineering is responsible for the entire NIEHS facility currently comprised of 192,671 square feet of leased space and 452,273 square feet in government-owned facilities on the South Campus site. The North Campus principal interim site provides 154,000 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 under construction are on a 509-acre tract of land in the Research Triangle Park that is in close proximity to the interim facilities. The new facilities, designed to NIEHS requirements, were funded by a $67 million Congressional appropriation. A construction management firm was selected in December 1976; construction contracts were subsequently awarded and construction began in April 1977. The shops building, warehouse, power plant and administrative modules A and B of Building 101 have been completed, accepted by NIEHS and occupied. Construction delays have resulted in a revised completion date of early 1982 for the laboratory modules. Upon occupancy of the South Campus facilities, NIEHS will continue to occupy the principal current facilities, while "off site" leased facilities will be relinquished.

The table below outlines the function and amount of space in both the current and new 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 (includes 8,097 sq.ft. off site)	69,155	129,640
Animal	24,353	81,860
Biostatistical Labs	4,148	20,460
Direct Lab Support	7,750	37,880
Office (includes 4,460 sq.ft. off site)	28,250	25,380
Conference Facilities & Public Space	2,335	7,180
Cafeteria	2,090	12,070
Library	3,650	0
Other	0	19,530
Subtotal, Program Facility	141,731	334,000
Support Services		
Power Plant & Incinerator	19,580	51,706
Shops	3,994	29,883
Warehouse	27,366	26,935
Communications & Electrical	0	9,749
Subtotal, Support Services	50,940	118,273
Total	192,671	452,273

Office Functional Units: The Office of Facilities Engineering is divided into four functional units in addition to the Office of the Chief. The Resource Management Section is the coordination point for all Office service requests providing planning/estimating, maintenance scheduling, material expediting, and shops materials and parts storage and disbursement services to the Office. 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 Services Unit provides shops, instrument fabrication and repair, and electronics repair services to the Institute. Contracts for janitorial and security services, grounds maintenance, major equipment preventive maintenance and repair, and operations of leased facilities are managed by this unit. The Office of Facilities Engineering also provides arts, graphics and

photography services to Institute personnel as adjuncts to publications of papers, conferences, seminars, and scientific exhibits.

Goals and Accomplishments:

Fiscal Year 1981 has been a period of reorganization and growth for the Office of Facilities Engineering directly related to its expanded mission in Facilities Operations and the initiation of operations on the South Campus. Of prime importance was the identification, recruitment and hiring of professional and technical staff to provide adequate management leadership, engineering expertise and operation skills to meet the new requirements of the Institute. The hiring freeze implemented by President Carter in February 1980 and continued by President Reagan in November 1980 created difficulties in meeting staffing requirements. It was only through an exception to the hiring freeze granted by HHS Secretary Patricia Harris in July, 1980 that adequate staff were hired to fill key positions and begin operation of the South Campus power plant in December 1980. Selection of Mr. Sirio Flores as Office Chief, and filling key engineer positions in the Engineering Design section and operator positions in the Facilities Operations Unit were major accomplishments for the Year.

The assumption of utility operations in the South Campus Power Plant and the occupation of Modules A and B of Building 101 were also major Office accomplishments in 1981. After months of planning and coordination between OFE personnel and other Institute Administrative and Management staff, the offices in Modules A and B of Building 101 were accepted by the Government in March, 1981. The Office of the Director, sections of the Office of Administrative Management, the Office of Health Hazard Assessment, the Office of Program Planning and Evaluation and portions of the Biometry and Risk Assessment Program moved into their new quarters in early April. Immediately following the moves into Building 101, OFE began renovations to vacated offices on the North Campus, preparing them for backfilling by offices currently housed in off-site leased quarters. Other planned renovations to North Campus laboratory buildings continued through the year and anticipated third and fourth quarter contract awards should complete all major renovations budgeted for in Fiscal Year 1981.

In addition to the more visible operations, renovations and acceptance of new facilities, the Office of Facilities Engineering has expanded its services to the Institute through the consolidation, award, and administration of several major operations, maintenance and service contracts. The Office spent $1,400,000 in fiscal year 1981 to deliver services which range from equipment repair and preventive maintenance to security, custodial, grounds maintenance and custom photographic processing.

The goals outlined above were accomplished despite significant problems. In addition to the hiring freeze already discussed, the Office had difficulty locating and recruiting the specialized staff it required. This in turn caused a delay in the delivery of services and in the development of internal management procedures and guidelines.

Future Objectives: Many of the OFE objectives for Fiscal Year 1982 are continuations of 1981 goals. The primary objective is the recruitment and hiring of sufficient personnel to complete the first phase of the Office reorganization and staffing plan. The Office, with vacancies in key positions in all of its

sections, is currently seven positions below its 1980 budgeted ceiling of 64. An additional 15 positions are needed to assure the uninterrupted delivery of services to the Institute and eliminate the extensive overtime now required in the Facilities Operations Unit.

The Engineering Design Section will be devoting 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 upgrade deteriorating buildings and systems and to prepare for further backfilling by NIEHS personnel. The modifications and completions on the South Campus will be confined to those changes which are an absolute prerequisite to the functional occupancy of a laboratory or to provide services for the installation of special bench/casework or free-standing equipment on the bare wall present in most C, D, and E laboratory modules.

Office management personnel will continue to work closely with DHHS, ROFEC staff to reach an orderly and timely completion of the NIEHS South Campus facility. The Facilities Operations Unit will develop an overall strategy for the operation and maintenance of the South Campus facility and integrate it with the ongoing activities of the North Campus.

SAFETY OFFICE

SAFETY AND HEALTH PROGRAM
Summary Statement

The Safety and Health Program has broad responsibility for employee health, chemical radiation, and physical safety. The program presently operates from two offices: one responsible for radiation safety; and, a second office responsible for chemical and physical safety and occupational health. These activities are being merged into a safety and health office with a manager with overall responsibility.

Routine duties of the Chemical Safety Office include quarterly surveys of Institute facilities, a limited air sampling program for selected work place contaminants, quarterly laboratory hood surveys, noise surveys, processing protocols for hazardous chemical use, and the pick up and disposal of hazardous materials.

Disposal of hazardous materials continues to be a national problem as evidenced by new regulations promulgated under the Resource Conservation and Recovery Act of 1976 (RCRA). The lack of a high temperature incinerator makes it necessary for NIEHS to landfill wastes that could be thermally degradated. Investigations are currently underway to determine the best design for such an incinerator that would be located on the South Campus. Additionally, a design for a new waste handling facility for both radioactive and hazardous wastes has been submitted. It is hoped that the structure will be completed to coincide with the opening of the South Campus laboratories.

The NIEHS Safety and Health Manual is in the process of being revised and updated. It is anticipated that the revisions should be completed and issued by January 1, 1982.

The Radiation Safety Office is concerned with providing for the environmentally safe use of radioisotopes at NIEHS for employees, visitors, and the surrounding community.

Routine duties of the office take most of the time of the radiation safety personnel. These 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, pick up and disposal of wastes, bioassay procedures, delivery and receipt of personnel dosimeters, and keeping an inventory of all radioisotopes at the Institute. The duties also include keeping accurate and detailed records for the items listed above.

The amount of radioactivity used at the Institute continues to increase with 1219 mCi of activity being received on site in 1978, 3777 mCi in 1979, and 8274 mCi in 1980. This activity is in addition to 24,000 Ci of Cs-137 which will be used in an irradiator facility at NIEHS.

In addition to routine duties, the Radiation Safety Office has been involved with the Biometry Branch in developing a computerized system utilizing local climatic conditions to predict radionuclide concentrations in air and doses due to incineration of radioactive material.

Disposal of low level radioactive wastes continues to be a national problem and has resulted in many new regulations and procedures for disposal. At present, there is only one site that will take wastes from NIEHS due to technical legal problems. In addition, the political climate makes it clear that this one site can be closed with only a few days notice. Since NIEHS is licensed to incinerate some radioactive material, the Radiation Safety Office is continuing to investigate acceptable methods of incineration and monitoring procedures to insure that the environment is not degraded.

LIBRARY

LIBRARY AND INFORMATION SERVICES
Summary Statement

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include manual and computerized literature searching of more than 200 bibliographic data bases, maintenance of a collection of some 665 periodical titles and 7,000 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 2040 new books for the Library and the laboratories, publication of a monthly newsletter, and compilation of the annual bibliography of publications by Institute personnel.

Reference/Literature Searching: The Library maintains one of the most up-to-date computerized literature searching capabilities in the world, with access to more than 200 data bases covering subjects from toxicology through business administration. During FY 81, Library personnel performed searches on 1,000 topics, usually using several data bases per question to ensure complete coverage. The number of regularly-scheduled current awareness searches (SDI's) continued to increase, but the printed copy of Current Contents remained the principal alerting service used by Institute personnel. The most heavily used data bases were TOXLINE, MEDLINE, Toxicology Data Bank, Biological Abstracts, and Chemical Abstracts.

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

The Library subscribed to 145 new serials during FY 81, bringing the total to more than 665. This large increase was due to placing a number of books in series on standing order via subscription. In addition, the Library ordered about 260 subscriptions for the various laboratories. The Library continued the policy of selectively binding journals or replacing them with microfilm to save space. By participating in several exchange programs, many missing issues were replaced. The collection now includes 9,000 journal volumes (17% bound) and 1,300 microfilm reels.

The NIEHS Library, working with EPA, produced a computer-generated journal holdings list updated for 1981.

Book Collection: Continuing the development of the book collection, 2040 books were purchased by the Library in FY 81. Of these, 43% were purchased for the Library and 57% for the Branches. Improvements were made in the computerized on-order file.

Through the Federal Library Committee, the Library continued using the automated cataloging system, OCLC, a computerized union catalog of books held by more than 1,000 libraries nation-wide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for new books which already have cataloging data on OCLC. At a push of the button the technician can register a new book on the system. In another joint project with EPA, the NIEHS Library developed a system to process the OCLC data into computer printouts which take the place of the card catalog. This computer-generated catalog contains title,

author, and subject indexes for the entire NIEHS book collection. In the future, this system will present the capability of maintaining an online catalog for looking up books via computer terminals in the Library and in the laboratories.

Interlibrary Loan: There was a 10% increase in the number of photocopy and book requests bringing the total to 15,730 in FY 81. Fortunately, as in the previous year, 43% were filled from the in-house collection. The student photocopying position at the Duke Medical Library was not renewed this year, but an arrangement was made with a company to provide document delivery services. This company provided about 25% of the total requests. This left about 30% of the requests which had to be sent to other libraries both in and outside the U.S.

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

Institute Manuscripts and Bibliography: The Library continued to maintain the NIEHS archives of manuscripts submitted for publication. Lists of new ones were published in the monthly newsletter. The Library published the 1980 NIEHS Bibliography, a catalog of the papers published by Institute personnel since 1966. The Bibliography, which included author and keyword indexes, was distributed to NIEHS authors and to interested parties in other government agencies and in industry.

Planning: Planning for library and information services to meet the needs of an expanded organization and new facility continued in FY 81. An architectural program of requirements and cost estimates was prepared for the construction of a new library in the basement area of A wing in the South Campus facility. A more spacious Library centrally located will provide better service to Institute personnel. Although actual construction is a long way off, the plan has been submitted to NIH. Meanwhile, the expansion of Library facilities in Building 18 began with the transfer of the Interlibrary Loan Office and Cataloging and Acquisitions Office into new space. Also, a major rearrangement of the bookshelves was undertaken to make better use of stack space.

The Librarian continued investigation of an integrated system for the automation of library functions. Two factors delayed a decision: the contract for a system proposed by the NIH Library was not put out for bids, and the general NIH freeze on data processing equipment prevented any procurement. In the meantime, several micro-computer systems directed toward libraries were placed on the market, and these systems appeared to offer an inexpensive, efficient option. The Librarian examined these systems at the annual meeting of the Special Libraries Association.

Close contact with various library and information organizations was maintained by NIEHS in FY 81. The Librarian was nominated to be Chairman of the Special Libraries Association (SLA) Environmental Information Division. He also served on the nominating committee of the North Carolina Chapter of SLA, and he continued serving as Business Manager of the N.C. SLA Bulletin. At the winter meeting of the N.C. Online Users Group of information specialists, the Librarian presented a paper on developing a system for computer-generated library catalogs.

INTRAMURAL RESEARCH PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR

LABORATORY OF ANIMAL GENETICS

LABORATORY OF ANIMAL GENETICS
Summary Statement

The focus of research done by members of the Laboratory of Animal Genetics is on the nature of genes and how they function, with the purpose of understanding mutation events and their impacts on reproduction and development in multicellular organisms. The effect of environmental agents on the genetic apparatus can be fully understood only if the molecular structure of genes and the details of gene activity and regulation are known. Recently the development of techniques for purifying genes and characterizing them in molecular terms has brought some startling developments and promises of answers to some of the most basic questions in genetics.

Eukaryotic Gene Structure and Regulation: Using Drosophila melanogaster as the model system, this group is concentrating on the structure of specific genes that show interesting features in their genetic organization and regulation. It has been discovered that the regulation of the white locus is controlled in part by another gene, zeste, rather tightly linked to it. Mutations of the zeste locus have the ability to repress white locus function when there are two copies of white arranged in their normal positions. Upsetting the position of the two white alleles relative to one another makes repression by zeste ineffective. Mutations in a specific part of the white gene also nullify the zeste action.

An intriguing aspect of the white gene regulation is the interaction that occurs between the two alleles in homologous chromosomes - an interaction termed transvection. Alleles appear to have some means of communication with each other, which is upset by chromosomal rearrangements that interfere with the pairing of homologous chromosomes. Studies suggest that this communication may be by means of a short-lived molecular species in low abundance. Experiments to test whether this is the case center on the identification and characterization of the RNA transcript from the white locus. The question is whether the locus produces a transcript, in addition to an mRNA that encodes protein, that is used in the nucleus as a signal in the regulation processes. The cloning of the white locus using an innovative method outlined in last year's report has provided the probes that allow this search. In the early phases of the study a 2 kb polyadenlylated RNA species has been identified in standard strains with transcripts of different size being found in a white mutant strain. Another approach to understanding the transvection mechanism is through the study of a series of mutant alleles whose expression is pairing-dependent. Their structures and the nature of their transcripts are being examined.

The structural organization of the white locus in molecular terms has proceeded rapidly. One of the most important discoveries is that many if not most of the spontaneously occurring mutants in this locus result from the insertion of non-white-locus DNA sequences into the locus. Some of these insertion mutants produce a phenotype equivalent to the null state of locus activity while others, curiously, retain a rather high level of gene function as judged by eye-color phenotype. This latter class suggests that the insertions are outside of the protein coding sequence yet perturb gene action possibly by upsetting regulation of the locus.

The study of hybrid dysgenesis, which is characterized by a 10-1000 fold increase in mutation rate among the progeny of crosses between certain Drosophila strains, shows that mutations at the white locus generated by this system are also associated with insertions of non-white-locus DNA into the locus. These discoveries call for revision of some of our views about the mutation process and open a set of new questions concerning the nature, origin and mobility of the insertion sequences. The sensitivity of the insertion sequences to environmental factors is not known but such information is necessary if we are to understand fully all aspects of environmental mutagenesis. Important implications concerning the basis for spontaneous mutations in other species, including humans, also grow from these discoveries.

Genetic Control of Transcription: A major step in gene expression is the transcription of DNA to form RNA, catalyzed by one of the RNA polymerases. RNA polymerase II , intrumental in the transcription of genes encoding proteins in higher organisms, is a heteromultimer composed of about 10 protein subunits. This enzyme complex and the genes that control it are under investigation in an effort to understand the control of the transcription process, how the multimeric protein interacts with DNA to recognize active genes from inactive ones, and how the complex interacts with other cellular factors influencing the specificity of transcription.

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

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

High titer antisera to the RNA polymerase II complex and individual subunits have been obtained and they are being used to identify the C4 gene product. If the genetic screens for identifying genes encoding other subunits fail, the antisera can be used to precipitate polysomes translating the nascent polypeptide chains. The RNA obtained this way may allow the cloning genes encoding other subunits.

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

The amino acid sequence of lactate dehydrogenase specific to the testis of the mouse (LDH-X) has been completely determined and 84% of the 330 residues of the

rat LDH-X has also been sequenced. Ten percent of the 330 residues differ between the two LDH-X types, most of which can be accounted for by single nucleotide changes. Two thirds of the differing residues are positioned on the surface of the molecules and may be involved in the antigenic determinants unique to the mouse and rat LDH-X isozymes.

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

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

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

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

Initial phases of the program have been a survey of the DNA composing the alcohol dehydrogenase locus (Adh) and adjoining regions in Drosophila melanogaster and related species. Restriction endonucleases were used to digest total genome DNA and the fragments produced were analyzed by a blotting technique that uses labeled DNA from a cloned Adh gene as a probe. The fragments that hybridize to the probe were then identified and their sizes determined. The pattern of restriction endonuclease sites were reconstructed to form a molecular map of the gene and adjoining regions. The data collected show a large amount of variation

consisting of insertions or deletions of DNA sequences flanking the structural gene for alcohol dehydrogenase. Since all of the Drosophila lines surveyed showed normal alcohol dehydrogenase activities, it is not clear what effect, if any, the insertion/deletion variations have on gene function. Other loci are under investigation to extend these findings and determine the general pattern of variation. As the molecular characterization becomes more complete, it is expected that variations in sequence arrangement and base pair differences can be evaluated in terms of the effects on gene expression.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60058-05 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Amount and Effects of Null Allozymic Variation in Natural Populations

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

PI:	C.H. Langley	Research Geneticist	LAG	NIEHS
Other:	R.A. Voelker	Research Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Drosophila melanogaster populations from North Carolina and Great Britian were sampled to determine the frequency of null alleles at 25 allozyme loci. Nulls were found at 14 of 20 autosomal loci with a weighted mean frequency of 0.0024, with a range from 0.00 to 0.01. No nulls were found at five X-chromosome loci. The frequencies and between locus comparisons suggest that null alleles are maintained in mutation selection balance. The data also indicate that allozyme loci are not characteristic of the whole genome.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Extraction of chromosomes from widely separated natural populations utilizes dominantly marked balancer chromosomes. Electrophoretic assays utilize gel electrophoresis and staining techniques. Cytological analyses involve examination and photography of salivary gland chromosome preparations. Immunologic assays involve preparations of antibodies to purified wild-type enzymes and detection of cross-reacting materials in mutants.

MAJOR FINDINGS AND PROPOSED COURSE: Quantitative and qualitative properties of null alleles (inactive) of 25 enzymes were similar in samples from two natural populations of Drosophila melanogaster (North Carolina and London, England). No null alleles were recovered from X-linked loci (5 in number). 57 autosomal nulls were distributed nonrandomly over the 14 loci but in a similar fashion in the two populations. The mean frequency ($q = 0.0024$) combined with independent mutation rate estimates and the similarity of unrelated populations indicate that these null alleles are in mutation-selection equilibrium with the average null depressing the fitness of the carrier by approximately 0.001. Further analysis also indicates considerably more genetic variation in these enzyme loci than most genes of the Drosophila genome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to assess the consequences of environmental mutagenesis, it is necessary to understand the quantitative and qualitative deleterious mutants present in a population. These surveys constitute the first study of this type of variation at specific loci where the genetic and biochemical nature of the mutants can be assessed.

PUBLICATIONS

Langley, C.H., Voelker, R.A., Leigh Brown, A.J., Ohnishi, S., Dickson, B., and Montgomery, E. Null allele frequencies in natural population of Drosophila melanogaster. Genetics (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60062-05 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Characterization of Isozymes and Mutant Enzymes in Mammals and Drosophila

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

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Others:	Y.-C. E. Pan	Visiting Associate	LAG	NIEHS
	F. S. Sharief	Biologist	LAG	NIEHS

COOPERATING UNITS (if any)

Departments of Biochemistry and Genetics, North Carolina State University, Raleigh, North Carolina

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

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

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

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

PUBLICATIONS

Okabe, M., Pan, Y-C. E., Sharief, F.S., and Li, S.S-L.: Molecular evolutionary relationship of mammalian lactate dehydrogenase isozymes, A4 (muscle), B4 (heart) and C4 (testis). Genetics, 97, S81, 1981.

Niesel, D.W., Pan, Y-C.E., Bewley, G.C., Armstrong, F.B. and Li, S.S-L.: Structural analysis of the soluble sn-glycerol 3-phosphate dehydrogenase isozymes and allelic variants of Drosophila melanogaster. Genetics, 97, S80, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60098-02 LAG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Amino Acid Sequences and Antigetic Structure of Mammalian Lactate Dehydrogenase Isozymes.

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

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Others:	Y.-C. E. Pan	Visiting Associate	LAG	NIEHS
	M. Okabe	Visiting Fellow	LAG	NIEHS
	F. S. Sharief	Biologist	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Lactate dehydrogenase isozymes from mouse testis and rat testis have been fragmented into small peptides by chemical and enzymatic cleavage. Most of these peptides have been separated and their amino acid sequences determined. Thus far, 100% of mouse LDH-X and 84% or rat LDH-X (330 amino acids in each protein) have been sequenced. Immunological properties of LDH-X isozymes from mouse and rat have also been characterized.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

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

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

PUBLICATIONS

Pan, Y.-C.E., Huang, S., Marciniszyn, J.P., Jr., Lee, C.-Y., and Li, S.S.-L.: The preliminary amino acid sequence of mouse testicular lactate dehydrogenase. Hoppe-Seyler's Z. Physio. Chem. 361: 795-799, 1980.

Pan, Y-C.E., Okabe, M., Sharief, F. and Li, S. S-L.: The amino acid sequences and immunological properties of testicular lactate dehydrogenase isozymes from rat and mouse. Fed. Proc. 40: 1886, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60099-02 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protein-Nucleic Acid Interactions and Organization-Regulation of Mammalian Genes

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

PI:	Steven S.-L. Li	Research Geneticist	LAG	NIEHS
Others:	K. Fong	Senior Staff Fellow	LAG	NIEHS
	Y.-C. E. Pan	Visiting Associate	LAG	NIEHS
	F. S. Sharief	Biologist	LAG	NIEHS
	M. Okabe	Visiting Fellow	LAG	NIEHS
	K. Akai	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.2	0.9

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Approximately 85% of the 333 residues from the gene 32 DNA-binding protein of bacteriophase T4 has been sequenced. The in vitro translated LDH-X of mouse testis poly A-RNA was identified by specific immunoprecipitation using rabbit anti-LDH serum.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purified proteins were fragmented into small peptides by CNBr, trypsin, chymotrypsin, and Staphylococcus V8 protease. The amino acid sequences of these purified peptides after gel filtration and ion-exchange chromatography were determined by automatic protein/peptide sequencer.

The total RNA was extracted by phenol/chloroform procedure from the testes of mature mice (16 weeks old), and poly(A)-containing RNA was obtained by passing the total RNA over oligo(dT)-cellulose twice. The functional poly(A)-RNA was translated in cell-free protein-synthesing systems from both rabbit reticulocyte and wheat germ lysates. The in vitro translated LDH-X was immunoprecipitated by anti-LDH-X serum followed by goat anti-rabbit IgG antibodies.

MAJOR FINDINGS AND PROPOSED COURSE: The amino acid sequences of approximately 85% of the 333 residues from T4 gene 32 protein have been determined. The unique characteristics of their amino acid sequences have been correlated with their functions.

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

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chemical structure of the DNA-interacting T4 gene 32 protein has elucidated how these proteins interact with single-stranded DNA as well as how T4 gene 32 protein functions in DNA replication, genetic recombination and repair of damaged DNA.

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

PUBLICATIONS

Pan, Y.-C.E., Nakashima, Y., Sharief, F.S., and Li, S.S.-L.: Amino acid sequence studies on T4 gene 32 DNA binding protein. Hoppe-Seyler's Z. Physiol. Chem. 361: 1139-1153, 1980.

Okabe, M., Fong, K., Tiano, H.F., Carter D.B., and Li, S.S.-L.: Cell-free translation of poly(A)-RNA from mouse testis and tentative identification of sperm-specific lactate dehydrogenase. Genetics, 97, S80-81, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61004-03 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Surveys of Genetic Variation Utilizing Two-Dimensional Electrophoresis

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

PI:	C. Langley	Research Geneticist	LAG	NIEHS
Others:	R. Voelker	Research Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Two-dimensional electrophoresis as described by O'Farrel in 1975 has been applied to the identification and estimation of genetic variation in various animal species. After surveying two Drosophila species, mice and humans for electrophoretic variation in the most abundant proteins, it can be concluded that previous estimates of the naturally occurring levels were overstated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Two-dimensional electrophoresis was carried out on either whole Drosophila or kidney tissue from mice or autopsied humans. Two species of Drosophila, various laboratory and wild populations of mice and twenty-five humans were surveyed for electrophoretic variation in the most abundant proteins. In the case of Drosophila and mice, the genetic variation was analyzed in crosses to demonstrate the modes of inheritance and linkage relationships.

MAJOR FINDINGS AND PROPOSED COURSE: The general conclusion of these studies is that the level of variation revealed by two-dimensional electrophoresis is less than that observed by earlier allozyme/isozyme techniques. Although there may be some sensitivity differences between the techniques, it seems more likely that biases in the selection of loci surveyed in previous studies accounts for the discrepancy. Although the amount of intraspecific variation detected in abundant proteins is lower than for allozymes in D. simulans and D. melanogaster, the genetic distances between the two species based on the variation estimates by the two procedures are comparable. The survey of genetic variation with two-dimensional electrophoresis will be concluded this year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The estimation of the standing levels of genetic variation and the understanding of their relationship to changes in the mutation rate is fundamental to the evaluation of public health risk.

PUBLICATIONS

Ohnishi, S., Leigh Brown, A.J., Voelker, R.A. and Langley C.H.: Estimation of genetic variability in natural populations of Drosophila simulans by two-dimensional and starch gel electrophoresis. Genetics (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61005-02 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetic Analysis of RNA Polymerase II Function in Drosophila melanogaster

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

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

COOPERATING UNITS (if any)

Dr. A. Greenleaf, Department of Biochemistry, Duke University, Durham, NC

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This study was initiated to determine the structure:function relationship of RNA polymerase II of Drosophila melanogaster. This enzyme is a heteromultimer consisting of approximately ten different subunits, each of which is presumably specified by a different locus. To date one locus, a mutant (C4) of which confers α-amanitin resistance, has been identified. We plan to clone C4 to determine which subunit of the enzyme is specified by C4. The cloned segment of the C4 locus will be used to study the molecular organization of that section of Drosophila X chromosome to see if genes coding for other subunits of RNA polymerase II reside there. To broaden our scope of understanding of transcription by RNA polymerase II, we also plan to use this system to identify non-polymerase II components of the transcription apparatus.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: A locus which confers α-amanitin resistance to RNA polymerase II has been identified. The locus is lethal-mutable. Different alleles at the locus affect male fertility and act as an enhancer of alleles at the other loci. To date, we have recovered at least 8 temperature-sensitive mutants. Additionally, a number of incompletely characterized mutants show promising results. Drosophila stocks (strains) that are essential to the recovery of suppressors have been constructed. We will use these specially synthesized strains to identify genes coding for other elements of RNA polymerase II structure and function. Attempts will be made to clone the α-amanitin resistant allele (C4) by recovering mutants induced by insertion of a transposable element.

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

Greenleaf, A. L., Weeks, J. R., Voelker, R. A., Ohnishi, S. and Dickson, B.: Genetic and biochemical characterization of mutants at an RNA polymerase II locus in D. melanogaster. Cell 21: 785-792, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61006-02 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

The molecular genetics of the w^a mutation of the white locus of Drosophila melanogaster

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

PIs:	Paul M. Bingham	Staff Fellow	LAG	NIEHS
	Burke H. Judd	Chief	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We have shown by fine scale genetic analysis and in situ hybridization that homology to the copia transposable DNA sequence element maps to the central portion of the white locus in the w^a allele at white. This homology to a transposon and this mutant allele are very tightly linked and we speculate that the w^a mutation results from the insertion of the copia transposon into the central portion of the white locus.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: We have employed fine scale genetic analysis as well as *in situ* hybridization to Drosophila polytene chromosomes.

MAJOR FINDINGS AND PROPOSED COURSE: Our results strongly support the hypothesis that the w^a mutation at the white locus results from the insertion of the *copia* transposon. Our results constitute the first direct demonstration in multicellular organisms that transposons make mutations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Transposons may represent an extremely important source of mutation in all multicellular organisms. Moreover, tumor viruses in mammals and birds have proven in numerous cases to be transposons. The analysis of transposons in multicellular organisms is one of the critical areas of research in both mutation and cancer.

PUBLICATIONS

Bingham, P. M., and Judd, B. H.: A copy of the *copia* transposable element is very tightly linked to the w^a allele at the white locus of *Drosophila melanogaster*. Cell (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61008-02 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical and Genetic Analyses of a Mutable Allele at the White Locus of *Drosophila melanogaster*

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

PI:	Paul M. Bingham	Staff Fellow	LAG	NIEHS
Other:	Zuzana Zachar	Guest Worker	LAG	NIEHS

COOPERATING UNITS (if any)

Duke University, Durham, NC

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We have characterized the physical nature of the w^{DZL} allele and some of its derivatives at the white locus of *D. melanogaster* in considerable detail. The basis for this mutation is the insertion of a large piece of DNA near the locus. Reversions of w^{DZL} are associated with partial loss of the transposable sequence.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: The following statements may be made on the basis of the analysis to date: The w^{DZL} mutation results from the insertion of a 12-14 kb DNA sequence element at the righthand (centromere-oriented) end of and probably outside of the white locus. This element has the capacity to mediate the rearrangment of contiguous chromosomal sequences in precisely the fashion expected if this inserted element is a transposon. The inserted element is homologous to sequences reiterated in the D. melanogaster genome, but the element does not exist, intact, at many (if any) other chromosomal sites. We speculate that it is a "scrambled cluster" element rather than a "copia-like" element.

We have further demonstrated that reversion of the w^{DZL} allele (to w^{+}) is associated with the deletion of portions (4-8 kb) of the element but not of the entire element.

Taken together these observations lead us to propose that the dominant mutant w^{DZL} allele results from the insertion of a transposable DNA sequence element outside of and to the right of the white locus. We speculate that the transposon produced the dominant mutant allele by promoting illicit transcription of the righthand portion of the white locus. We are currently testing this and other speculations concerning the molecular basis of w^{DZL} dominance.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We expect these studies to contribute to the understanding of the regulation of white locus expression and, thus, to the understanding of the regulation of expression of genes in multicellular organisms in general. Such an understanding is critical to the solution of both mutation and cancer problems. The phenomenon of hybrid dysgensis may be responsible for much or most of the spontaneous mutations arising in natural populations of Drosophila melanogaster. Comparable phenomenon may occur in other species, including humans, and the analysis of the phenomenon in Drosophila is expected to define the approaches for its analysis in other organisms.

PUBLICATIONS

Bingham, P. M.: The regulation of white locus expression: A dominant mutant allele at the white locus of Drosophila melanogaster. Genetics 95: 341-353, 1980.

Bingham, P. M.: A novel dominant mutant allele at the white locus is mutable. Cold Spring Harbor Symp. Quant. Biol. 45: 519-525, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61010-02 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Number, Size and Arrangement of Genes in Drosophila melanogaster

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.0	0.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The project focuses on the genetic and cytological organization of a small segment of the X chromosome of Drosophila. The objectives are to obtain mutant representatives of every gene in the region extending from 3A to 3C of the polytene map. Mutants are then characterized by complementation, recombination mapping and positioning relative to chromosomal aberrration break points. Gene numbers and sizes are determined. Those chromosomal segments in which breakage may occur without causing detectable modifications of gene function are also mapped.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED; Chromosomes were mutagenized by treating males with x-rays or one of a variety of chemicals. Mutants were recovered using a deletion for 3A-3C as a screening system. Characterization of mutants was by recombination complementation and mapping by rearrangements. Cytological examination of polytene chromosomes was by phase microscopy of smears of salivary gland cells.

MAJOR FINDINGS AND PROPOSED COURSE: The initial discovery that there exists a one-to-one relationship between chromomeres of polytene chromosomes and complementation groups has been pushed farther by searching for classes of mutations that were not easily recovered by the methods first employed. We have found exceptions to the one function-one-chromomere relationship but in general the close correspondence is maintained. The significance of this finding is that Drosophila genes may average as much as 25kb. We have also found several sites where break points in the segment we have saturated with mutations produce no detectable mutant effects. This aspect is being pursued further by creating additional rearrangements with break points in the 3A-3C segment and analyzing them for position and mutant characteristics. Proposed course is to analyze the region at the molecular level by cloning segments of the DNA and determining the size, number and organization of transcription units. During the last year this project has received very little effort because the appointment of a molecular biologist to extend this analysis has not been possible.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The genes of eukaryotic organisms appear to be very much larger than the messenger RNAs they generate. In some genes the sequence encoding a single protein may be interspersed with non-coding but transcribed regions that are removed in the maturation process of mRNA. It is important that we understand what a gene represents as a mutational target; how big it is; what changes can modify its function. We also must know how many genes there are in eukaryotic genomes if we are to understand the impact of environmental agents as mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61011-02 LAG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Regulation of Gene Function in Drosophila

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

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

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.25	1.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: We have concentrated on two loci in Drosophila that show different aspects of allelic interaction. The white locus exhibits some unusual characteristics in its regulation when homologous X chromosomes are paired vs. unpaired. The locus is repressed by the zeste mutation if two w^+ loci are paired or closely associated but both w^+ alleles are active if they are unable to pair. We have determined that it is the proximal segment of the w^+ locus that is important in this communication between alleles. The second locus under study is cut. Alleles at this locus show complementation between a group of alleles that modify the morphology of the wing and a group that modify the structure of the legs. Both groups fail to complement a cluster of lethal alleles that map at the proximal border of the locus. We are treating chromosomes carrying different ct alleles with x-rays and testing them for transvection effects. Several rearrangements have been recovered but none confer a transvection effect on the interaction of mutant alleles. Of particular interest are two mutations that appear to be due to break points located outside the locus itself. These appear to be stable position-effect changes but of a type that act only in cis to the cut locus.

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61012-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

The molecular basis of IR hybrid dysgenesis in Drosophila melanogaster.

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

PIs:	Paul M. Bingham	Staff Fellow	LAG	NIEHS
	Joseph W. Jack	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We are examining the molecular basis of the IR type hybrid dysgenesis in Drosophila melanogaster. Information about another dysgenic system (PM) implicates a family of transposable DNA sequences as the basis for the burst of mutagenic activity observed. We wish to determine whether a similar mechanism exists for the IR system.

PROJECT DESCRIPTION

METHODS EMPLOYED: We will make extensive use of both genetic techniques and the techniques of nucleic acid biochemistry.

MAJOR FINDINGS AND PROPOSED COURSE: Two systems of hybrid dysgenesis have been characterized in D. melanogaster, the PM system and the IR system. While these two systems show certain similarities they are clearly distinguishable from one another. Bingham, Rubin and Kidwell (unpubl.) have recently shown that the mobilization of a single transposon family may account for most of the properties of PM hybrid dysgenesis. Pursuant upon the analysis of these authors, we are undertaking to ask if IR hybrid dysgenesis might, as well, show such a simple pattern of behavior. We are also attempting in the course of these studies to use "transposon tagging" (Bingham, Levis and Rubin, submitted) with the transposon presumed to be responsible for IR hybrid dysgenesis to clone the cut locus DNA sequences from D. melanogaster.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61013-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

In Vivo transcription of the white locus DNA sequences in Drosophila melanogaster

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

PIs:	Joseph W. Jack	Staff Fellow	LAG	NIEHS
	Paul M. Bingham	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We are analyzing RNAs produced by several strains of D. melanogaster carrying various different white locus mutations and comparing them to RNAs from wild type strains. Probes for this analysis are labeled sequences of the white locus DNA that allow recognition of in vivo transcripts of the locus.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: We will employ the techniques of nucleic acid biochemistry and, implicitly, of genetic analysis.

MAJOR FINDINGS AND PROPOSED COURSE: To date we have shown that a 2 kb polyadenylated transcript exists that is homologous to sequences to the left (toward the telomere) of the *copia* insertion at w^a but not detectably homologous to sequences at and to the right of this site. This transcript is present at a level of *ca*. 1 picogram per 500-1000 pupae or adults. We are currently developing more sensitive methods for the detection of RNA sequences present in very small amounts and will use these techniques to analyze the pattern of transcription of the locus in more detail.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We anticipate that this and other studies will lead to an understanding of the regulation of expression of the white locus. This, in turn, may prove central to the understanding of the regulation of gene expression in multicellular organisms; such an understanding is important to both the problems of mutation and of cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61014-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
The DNA sequence of the white locus of D. melanogaster.

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

PIs:	Y.-C. E. Pan	Visiting Associate	LAG	NIEHS
	Paul M. Bingham	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We are currently sequencing the righthand (centromere proximal) portion of the white locus of Drosophila melanogaster.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: We are using conventional DNA sequencing techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The proximal portion of the white locus contains sites at which mutant alleles affecting the regulation of expression of the locus map. Our sequence data to date reveal several segments of DNA within this region of very high AT content (at least 70%). We are pursuing these studies and it is to be expected that the results will be critical to the analysis of the molecular basis of the regulation of white locus expression.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We expect these studies to contriubte to the understanding of the regulation of white locus expression and, thus, to the understanding of the regulation of expression of genes in multicellular organisms in general. Such an understanding is critical to the solution of both mutation and cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61015-01 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The structure and function of the region of very weak DNA sequence homology between the white locus and the zeste region in Drosophila melanogaster

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

PI:	Paul M. Bingham	Staff Fellow	LAG	NIEHS
Other:	Thaddeus Bargiello	Guest Worker	LAG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIH, NIEHS, Research Triangle Park, NC 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 (200 words or less - underline keywords)

Bingham, Levis and Rubin (unpubl.) found cloned segments in a hybrid phage clone library made from D. melanogaster DNA that showed very weak DNA sequence homology to the white locus region from this same organism. We have shown that most or all of these cloned segments derive from distal 3A on the polytene chromosome map (the region containing the zeste locus, a locus that interacts genetically with the white locus). We are currently analyzing this phenomenon in more detail.

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: Our results to date suggest that this zeste-region locus is complex and contains at least two separate regions of DNA sequence homology to the white locus. Further, the region within the white locus homologous to each of these sites in the zeste region is also the region in which insertion-generated mutations affecting the zeste-white interaction map. We speculate that the zeste and white loci interact because of DNA sequence homology, for example, by virtue of base pairing between a zeste locus transcript and a white locus transcript. We are currently testing this and other speculations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We expect these studies to contribute to the understanding of the regulation of white locus expression and, thus, to the understanding of the regulation of expression of genes in multicellular organisms in general. Such an understanding is critical to the solution of both mutation and cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61016-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Basis of PM Hybrid Dysgenesis in Drosophila melanogaster.

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

PI: Paul M. Bingham Staff Fellow LAG NIEHS

COOPERATING UNITS (if any)

M. G. Kidwell, Brown University, Providence, RI
G. M. Rubin, Carnegie Institution of Washington, Baltimore, MD

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PROJECT DESCRIPTION

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

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

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61017-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Physical map of the white locus and the nature of spontaneous white mutant alleles arising in laboratory strains of D. melanogaster.

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

PI:	Paul M. Bingham	Staff Fellow	LAG	NIEHS
Other:	Zuzana Zachar	Guest Worker	LAG	NIEHS

COOPERATING UNITS (if any)

Duke University, Durham, NC

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We have analyzed the DNA sequence organization of several white mutant alleles arising in laboratory stocks of D. melanogaster and collected by various other workers during the last 75 years. Some of the properties of the mutations have been determined and a physical map of the locus has been constructed.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: The following general statements can be made on the basis of the analysis to date indicates: a majority (6/8) of spontaneous, stable "point" mutant alleles at white are associated with the insertion of non-white-locus DNA sequence elements into the white locus DNA. Various properties of these mutations allow us to infer, in several cases, that the insertion associated with a mutant allele is in fact, responsible for the mutation in question. This, in turn, allows us to define the physical map of the locus and to map this, in turn, onto the genetic map of the locus. This mapping allows us to calculate that there are from 1.5 to 2.7 x 10^{-3} cM (recombination) per kilobase (DNA sequence length).

We have, further, characterized two local, tandem duplications of white locus DNA sequences that enhance the mutant phenotype of individuals carrying the z^1 allele at the zeste locus. The duplicated segments in these two alleles overlap by only 8-10 kb and this region of overlap contains the righthand (w^{ch} and w^{sp} sites) portion of the locus but not the remaining portions of the locus.

In overview, these studies suggest that a substantial fraction of spontaneous mutations arising in laboratory stocks result from the insertion of DNA sequence elements. These studies further provide the critical framework for other studies in this laboratory directed at understanding the molecular biology of the regulation of white locus expression.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We expect these studies to contribute to the understanding of the regulation of white locus expression and, thus, to the understanding of the regulation of expression of genes in multicellular organisms in general. Such an understanding is critical to the solution of both mutation and cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61018-01 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Population genetic variation in DNA sequences

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

PI: C.H. Langley Research Geneticist LAG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Variation in the restriction map in the Adh region (alcohol dehydrogenase locus) of chromosome II of Drosophila melanogaster and some related species were examined. Insertion/deletion differences outside the transcriptional unit were common. The distribution within Drosophila melanogaster and among the species suggest that such variants are deterious mutants, although they do not have any obvious effect on Adh function.

PROJECT DESCRIPTION

METHODS EMPLOYED: Genetically isolated second chromosomes of Drosophila melanogaster and various isofemale lines of related species were reared and nuclear DNA isolated. This was digested with various restriction enzymes, electrophesed, blotted (Southern) and probed with several cloned sequences from the Adh region. Restriction maps were constructed.

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61019-01 LAG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Collaborative DNA and Protein Sequencing

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The complete primary structure of the 90 residues of a seminal vesicles secretory protein (SVS-IV) from rat has been sequenced. Sequence information of about 95% of the 186 residues from human dihydrofolate reductase has been obtained. Various restriction sites and approximately 800 nucleotide sequence of a DNA of 3.5kb nucleotides from white locus of Drosophila have been determined.

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.

Nucleotide sequencing: restriction enzymatic digestion was used to cleave 3.5kb DNA into small DNA fragments. Those DNA fragments were purified by electrophoresis on polyacrylamide gel and/or agrose gel. Nucleotide sequencing technique as developed by Maxam and Gilbert was then used to obtain the nucleotide sequences of small DNA fragments.

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

Various restrictions sites on 3.5kb DNA of white locus from Drosophila have been located. Some of the small DNA fragments have been isolated and about 800 nucleotide sequence information was obtained. The complete DNA sequence of white locus will be obtained in the near future and the genetic regulation and function of white locus in relation to various mutant white loci will be studied.

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

PUBLICATIONS

Pan, Y-C. E., Silverberg, A. B., Harris, S. and Li, S. S.-L.: The complete amino acid sequence of a major secretary protein from rat seminal vesicle. Int. J. of Peptide and Protein Research 16: 143-146, 1980.

Pan, Y-C.E., and Li, S.S.-L.: Complete convalent structure of seminal vesicle secretory protein IV from rat. Fed. Proc. 39: 1998, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61020-01 LAG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

The cloning of the white locus DNA sequences from the w^a and Canton-S strains of D. melanogaster

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

PI: Paul M. Bingham Staff Fellow LAG NIEHS

COOPERATING UNITS (if any)
G. M. Rubin and R. Levis, Carnegie Institution of Washington, Baltimore, MD

LAB/BRANCH
Laboratory of Animal Genetics

SECTION
NIH, NIEHS, Research Triangle Park, NC 27709

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

DNA sequences of the white locus of Drosophila melanogaster have been cloned by a method we have developed referred to as "transposon tagging". The white locus allele, w^a, contains a copy of the copia transposable element inserted in a central portion of the white locus. This transposon has been used to recover a part of the white locus DNA in cloned form.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: We have developed a method referred to as "transposon tagging" that we expect to be generally applicable to the cloning of any gene in D. melanogaster identified by genetic analysis when this method is applied in light of the recent observations of Bingham, Rubin and Kidwell (unpubl.) regarding the molecular basis of PM hybrid dysgenesis. We have used this method to clone a segment of the white locus region from w^a strain on the basis of this allele possessing a copy of the copia transposable element DNA sequences inserted in the central portion of the white locus in this allele. We have walked from this original cloned segment to clone a ca. 50 kb interval from Canton-S and have shown, by placing on this cloned interval various chromosomal breakpoints bracketing the white locus, that the white locus is probably no larger than 12 kb and may be smaller. These studies have formed the initial ground work for subsequent studies by Z. Zachar and P. Bingham on the physical structure of the white locus and for other studies directed at understanding the molecular basis of the regulation of expression of the white locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: We expect these studies to contribute to the understanding of the regulation of white locus expression and, thus, to the understanding of the regulation of expression of genes in multicellular organisms in general. Such an understanding is critical to the solution of both mutation and cancer problems.

PUBLICATIONS

Bingham, P.M., Levis, R. and Rubin, G.M.: The cloning of DNA sequences from the white locus of Drosophila melanogaster using a novel and general method. Cell, in press, 1981.

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 and neurochemistry.

BEHAVIORAL TOXICOLOGY

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

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

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

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

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

Kepone, in a ninety day chronic dosing study of 30 ppm was found to increase a startle response to both an air puff and an auditory stimulus after 30, 60, and 90 days of dosing. Recovery of function was observed 30 days after cessation of dosing.

2,4-Dichlorophenoxyacetic acid (2,4-D) is being studied for its neurobehavioral toxicity in rats. This compound is a widely used herbicide and a component of Agent Orange. There have been scattered reports of delayed neuropathy following exposure to this agent. The consequences of exposure to 2,4-D on neurobehavioral functioning have not been studied systematically in animal models.

Several studies are in progress concerning the neurobehavioral toxicity of agents administered prenatally, neonatally, or both. These studies are being conducted in collaboration with other laboratories at NIEHS and/or the local universities. One such project determined the neurobehavioral effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl in mice. Offspring of mothers given the compound on days 6-13 of gestation were assessed for neurobehavioral deficits for up to one year after birth. It was found that, when tested in adulthood, these mice demonstrated signs of neurotoxicity. The most severely affected subjects displayed a neurobehavioral syndrome consisting of intermittent stereotypic circling, head-bobbing, hyperactivity, impaired forelimb grip strength, and impaired ability to traverse a wire rod. Some mice did not display the spinning syndrome but were found to be deficient in traversing a wire rod and in acquisition of an avoidance response. Thus, exposure to this polychlorinated biphenyl compound can influence neurobehavioral functioning of mice during adulthood and, in some cases, such effects can be

observed in the absence of clinical signs of toxicity. Another project concerned the effects of neonatal exposure to diethylstilbestrol and testosterone on the neurobehavioral functioning of rats at various times during development. It was found that high levels of estrogen and diethylstilbestrol present during the period of sexual differentiation feminize or demasculinize the male and this effect can be observed in adulthood using appropriate sexually dimorphic neurobehavioral and morphometric measurements.

Neonatal exposure (on days 9, 11, and 13) to 500 mg/kg of benzene administered subcutaneously in corn oil to rat pups was found to increase the spontaneous motor activity of these animals when tested at 100-130 days of age. In addition, the motor-stimulating effect of d-amphetamine was reduced in these animals.

A study concerning the effects of exposure to polybrominated biphenyls on neurobehavioral development in mice was conducted giving 3 or 10 mg/kg FF-1, by gavage, every other day during gestation until weaning at 21 days. Long-term alterations in the responsiveness of these mice to certain types of novel environmental conditions were observed.

Extensive studies concerning the developmental neurotoxicity in rats of acrylamide and triethyl tin are currently in progress. In addition, the long-term neurobehavioral effects of embryonic exposure of Japanese quail eggs to microwave irradiation is being investigated. Measures being studied are sexual and reproductive behaviors, a battery of behavioral and neurological measurements, learning and retention, and baseline performance on a multiple fixed ratio/fixed interval schedule. Preliminary results suggest a decreased reactivity to shock stimuli and a possible deficit in learning ability in birds exposed to 2450 MHz CW microwave radiation at a power density of 5 mW/cm^2.

NEUROCHEMISTRY

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

A series of high affinity cerebral binding systems are being developed with the purpose of utilizing this assay as a routine neurotoxicological test. Ligand-receptor interactions that have been characterized include those for serotonin, GABA, diazepam, glycine, muscarinic acetylcholine, dopamine, and the a and b adrenergic sites. Enkephalin receptors will also be studied. Regional distribution and specificity determinations have been made for these receptors. The developmental profile of receptors is being assayed in rats and chicks. The extent to which circadian and other environmental factors influence receptor formation and maintenance is under study. Specific increases in the striatal dopamine receptor have been found in acrylamide-treated rats after single or repeated dosing. However, prenatal or neonatal exposure to acrylamide results in a reduction of striatal dopamine receptors. This receptor is depressed in adult mice prenatally exposed to polychlorinated biphenyls. Kepone-treated rats exhibit more general receptor changes. Manganese and tin exposed rats are also being tested. Receptors have been shown to be inhibited *in vitro* by low concentrations of tri-n-butyl lead acetate but not by lead

acetate. Methyl mercuric chloride is generally less inhibitory than inorganic mercuric chloride.

The neuroendocrine and neuropeptide profile of toxically treated animals is being developed as a potential tool in the systematic evaluation of neurotoxicity. These assays are performed by radio-immune techniques involving the iodination of purified antibodies. Levels of prolactin, ACTH, TSH, and steroid hormones are determined in serum while endorphins, enkephalins, neurotensin, and substance P are assayed in homogenates of brain regions. Toxicants that have been tested include acrylamide, kepone, and monosodium glutamate. The neonatal administration of this latter compound has been found to significantly depress plasma testosterone levels of treated male rats. Kepone-exposed rats showed no significant alterations of testosterone or prolactin.

In addition, a variety of biochemical parameters will be tested to determine the extent to which they may provide useful reflections of animal exposure to neurotoxic agents and/or of animal behavioral modification by various drugs. Several enzymes will be used to provide markers for plasma membranal-, mitochondrial-, endoplasmic reticulum-, and nuclear function, as well as several aspects of energy and neurotransmitter metabolism. It is anticipated that a general biochemical screening technique will evolve to permit prediction of neurotoxicological consequences of animal exposure to toxic agents of environmental interest.

The effects of acrylamide on the metabolism of striatal dopamine is being studied from several aspects including assay of its levels, rates of breakdown, and receptor density in rats. This is being done because acrylamide poisoning is frequently associated with tremor and it is plausible that the locus of origin is the striatum.

The relative effectiveness of several alkyl lead compounds, inorganic lead, methyl mercury, and inorganic mercury as inhibitors of caudate nucleus adenylate cyclase activities has been investigated. Methyl mercury, inorganic mercury, and several alkyl lead compounds are relatively effective inhibitors of the dopamine-sensitive adenylate cyclase, whereas inorganic lead was not an effective inhibitor. Organic lead salts are of particular interest as inhibitors of the dopamine-sensitive adenylate cyclase, because those salts with hydrophobic substitients are more effective inhibitors than those with greater water solubility. For instance, tri-n-butyl lead acetate is more inhibitory than tri-n-propyl lead acetate, which is more inhibitory than triethyl lead acetate. From another point of view, the extent of aryl (phenyl) substitution of lead correlates in a positive fashion with the inhibitory effectiveness of the alkyl lead salt. The dopamine sensitive form of adenylate cyclase is usually inhibited at alkyl lead concentrations lower than those required for inhibition of basal adenylate cyclase activity.

PERSONNEL

Additions to the Laboratory were: Interagency Personnel Agreement - Dr. J. Rosecrans; Staff Fellow - Dr. C.F. Mactutus; Visiting Fellow - Dr. S.Y. Ali; Technician - N.J. Peterson. Individuals leaving the Laboratory of Behavioral and Neurological Toxicology were Visiting Scientist, Dr. P.K. Seth; Visiting Fellow, Dr. S.B. Por; and technician, M.T. Riley.

OTHER ACTIVITIES

Dr. S. C. Bondy: Adjunct Associate Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina; Member, Editorial Board, Developmental Neuroscience; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurochemical Research; Councillor, North Carolina Society for Neuroscience; Chairman, Session on Receptors, American Society for Neurochemistry, Richmond, Virginia; Contributor to Round Table "Neurotoxicology of Heavy Metals," American Society for Neurochemistry, Richmond, Virginia; Member, NIEHS Radiation Safety Committee; Member, Publications and Education Committee, American Society for Neurochemistry; Invited seminar entitled "Neurochemical Study of Toxicity in the Brain," Royal Postgraduate Medical School, London, England; Invited seminar entitled "Screening for Signs of Toxicity in the Brain," LaTrobe University, Melbourne, Australia.

Dr. P. A. Cabe: Adjunct Assistant Professor, Department of Psychology, University of North Carolina; Adjunct Assistant Professor, Department of Psychology, North Carolina State University; Member, NIH Working Group on Behavioral and Social Science Research Committee, NIEHS; Member, Research Triangle Neurotoxicology Seminar Series Steering Committee; Invited seminar entitled "Chemical Effects on Behavior," Sanderson High School, Raleigh, North Carolina; Invited seminar entitled "Behavioral Toxicology in Japanese Quail: Effects of Chemical and Physical Agents," NIEHS Institute Seminar Series; Invited presentation entitled "Laboratory Assessment of Somatosensory Organ Function," Target Organ Toxicity: Eye, Ear, and Other Special Senses, Alexandria, Virginia.

Dr. J. S. Hong: Adjunct Associate Professor, Department of Psychiatry, Duke University Medical School; Invited seminar entitled "Biochemical Studies of Neuropeptides on Rat Brain," Duke University Medical School; Invited seminar entitled "Brain Peptides as Neurotransmitters," Duke University Medical School.

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, Intramural Council, NIEHS; Councilor, North Carolina Society for Neuroscience; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial Board, Neurotoxicology; Member Editorial Board, Neurobehavioral Toxicology; Faculty Member, Neurobiology Curriculum, University of North Carolina; Invited seminar entitled "Overview of Behavioral Toxicology with Special Reference to Problems Associated with Data Analysis," Annual DRUSAFE Meeting, Sponsored by the Pharmaceutical Manufacturing Association, Tarpon Spring, Florida; Invited presentation entitled "Behavioral Toxicology in Safety Assessment," Symposium on Behavioral Toxicology, Society of Toxicology of Canada, Montreal, Canada; Invited seminar entitled "Behavioral Toxicology in Risk Assessment: Problems and Research Needs," Department of Pharmacology, University of North Carolina.

Dr. H. A. Tilson: Adjunct Associate Professor, Department of Zoology, North Carolina State University; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology; Invited lecturer in graduate level Neurotoxicology course, The George Washington University Medical Center, Department of Pharmacology, Washington, D.C.; Invited participant in the

Conference on Neurotoxicity of Organic Solvents co-sponsored by the Environmental Protection Agency and Johns Hopkins University School of Medicine, Raleigh, North Carolina.

Dr. L. L. Uphouse: Member, Animal Experimentation Committee, National Institute of Environmental Health Sciences; Ad Hoc Reviewer, National Science Foundation Neurobiology Program; Ad Hoc Reviewer, National Science Foundation Psychobiology Program; Ad Hoc Reviewer, National Science Foundation EPSCOR Program; Invited seminar entitled "Experimental Modulation of Brain Transcription," Dorothea Dix Hospital, Raleigh, North Carolina; Invited seminar entitled "Potential Models for the Neurotoxicity of Kepone," National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland; Invited seminar entitled "Holistic Approach to Mental Health Promotion: Role of the Genotype," Carolina's Primary Prevention Conference, Asheville, North Carolina; Invited participant in the "Mini-Course in Neurotoxicology," Department of Psychology, University of Colorado, Boulder, Colorado; Invited presentation entitled "Environmental Effects on Health with Emphasis on the Nervous System," Interagency Panel on Child and Youth, Washington, D.C.; Invited seminar entitled "Model of Neurotoxicity from Chlordecone," Dorothea Dix Hospital, Raleigh, North Carolina.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90008-04 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Acute and Chronic Neurobehavioral Toxicity of Acrylamide in Rats

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Acrylamide is an environmental toxicant capable of producing "dying back" central-peripheral axonopathies. Because acrylamide is representative of many neurotoxicants and neurological disease states, the profile of acrylamide neurotoxicity is being assessed in several behavioral procedures. The purpose of these studies is to standardize and validate testing procedures for the laboratory and to provide a basis for the study of neurotoxic mechanisms of action.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

A. Previous work in this laboratory suggested that acrylamide increases affinity and/or density of dopamine receptors in the corpous striatum (Agrawal et al., Toxicol. Appl. Pharmacol., in press, 1981). The present studies investigated the functional significance of the receptor binding effects. Rats were given a dose of 100 mg/kg of acrylamide orally and challenged with 1 mg/kg apomorphine 24 hours later. Apomorphine-induced hypermotility was significantly decreased by acrylamide pretreatment. A similar effect was observed after 10 days of dosing with 10 mg/kg/day.

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

   Rats were trained to lever touch for food on a variable interval (VI) 15 sec schedule of reinforcement. Dose response alteration of VI responding by apomorphine, d-amphetamine, clonidine, and chlordiazepoxide was studied alone and in the presence of a behaviorally ineffective dose of acrylamide. Pretreatment with 12.5 mg/kg of acrylamide 24 hrs prior to challenge with psychoactive compounds enhanced the behavioral suppressant effects of apomorphine and d-amphetamine. No significant effect of acrylamide pretreatment on the behavioral effects of clonidine and chlordiazepoxide was observed. These data suggest that acrylamide increases responsiveness to agents that act directly on dopamine (DA) receptors or indirectly by releasing DA. This change in responsiveness to apomorphine and d-amphetamine may be related to the effects of acrylamide on the affinity or density of the DA receptor.

C. Studies are underway to assess further the potential central effects of acrylamide using behavioral and psychopharmacological procedures.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The validation of neurobehavioral test methods is a crucial step in the development of neurotoxicological predictive capabilities. The studies described here provide an invaluable data base for further methods development, using a substance which in itself is a known environmental contaminant. The studies described are congruent with the Institute's mission to discover methods for the detection and prediction of environmental health hazards.

PUBLICATIONS

Tilson, H.A., Cabe, P.A., and Burne, T.B.: Behavioral Procedures Used in the Assessment of Neurotoxicity. In Spencer, P.S. and Schaumburg, H.H. (Eds.): Experimental and Clinical Neurotoxicology. Baltimore, Maryland, The Williams and Wilkins Company, 1980, pp. 758-766.

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90009-04 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Developmental Exposure of Japanese Quail to Microwave Irradiation

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

PI:	P. A. Cabe	Senior Staff Fellow	LBNT	NIEHS
Other:	D. I. McRee	Research Physicist	LEB	NIEHS
	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS

COOPERATING UNITS (if any)
Laboratory of Environmental Biophysics, NIEHS
Zoology Department, North Carolina State University
Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The neurobehavioral effects of embryonic exposure of Japanese quail (Coturnix coturnix japonica) to 2450 MHz CW microwave radiation at a power density of 5 mW/cm^2 during the first 12 days of incubation are under test. Previous work has suggested alterations in reactivity to shock stimuli and possible altered learning ability in exposed birds tested as adults. No effects on general health or spontaneous activity were found, however. Replication of these results is under study. Long-term assessments of sexual and reproductive behavior, sensory function, and other parameters are anticipated, and preliminary studies of such phenomena have been initiated. Drug challenge studies seem particularly likely to expose microwave effects; some preliminary studies with standard agents (d-amphetamine and haloperidol) have been done.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fertile Japanese quail eggs are exposed to microwave radiation (2450 MHz, CW, 5 mW/cm^2 incident power density) during the first 12 days of incubation. Hatchability, incomplete development, and any deformaties are noted.

Behavioral testing begins at 6-8 weeks. Activity in electronic activity monitors has been tested, followed by training in a shuttle-box shock escape-avoidance procedure. Food-deprived birds have been tested for acquisition of an autoshaped key peck response and ability to perform under operant schedules of reinforcement.

MAJOR FINDINGS AND PROPOSED COURSE: Indications of microwave-associated changes in shuttle-box performance and in autoshaping have been observed. No changes have been seen in general health, spontaneous activity, or performance on a random-interval operant schedule. Replication of these measures is currently in progress. Pilot work on naturally-occurring behavioral sequences is under way. Drug challenge experiments are planned with microwave-exposed birds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Microwave-generating devices are employed in a variety of military, industrial, and civilian applications. However, the consequences of prenatal exposure to microwave irradiation on neurobehavioral functioning have not been studied systematically, in spite of the known sensitivity of developing organisms to many types of chemical and physical insults.

PUBLICATIONS

Cabe, P.A. and McRee, D.I.: Behavioral teratological effects of microwave radiation in japanese quail (Coturnix coturnix japonica): An exploratory study. Neurobehav. Toxicol. 2: 291-296, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90011-03 LBNT

PERIOD COVERED
October 1, 1980 to January 1, 1981

TITLE OF PROJECT (80 characters or less)

Behavioral and Morphological Effects of Triethyl Tin in Adult Rats

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Triethyl tin (TET) is an environmental toxicant capable of producing demyelination of central and peripheral axons. Because TET is representative of many neurotoxicants, especially heavy metals, the neurotoxic profile of TET is being assessed in several behavioral procedures. The purpose of these studies is to assist in the development of a profile of known neurotoxicants which can be used to validate a battery of neurobehavioral tests.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The repeated administration of triethyl tin bromide (TET) (0, 1.0, 2.0, and 3.0 mg/kg, p.o.) to male rats produced dose- and time-dependent reductions in body weights and food and water consumption. Tests showed decreases in fore- and hindlimb grip strength and startle responsiveness. Histologic examination immediately after the two week dosing period showed that in all dose groups there was intramyelinic edema of major CNS white matter tracts, the severity of which varied according to the dose. Four weeks after cessation of TET dosing, body weights of the treatment groups had almost recovered to control group (0 mg/kg) values. There was complete recovery of food and water consumption. Retests for functional performance indicated complete recovery of all measures with the exception of continued reduction of startle responsiveness to an air puff stimulus. Histologic examination after the four week recovery period indicated that the 1.0 mg/kg dose group was indistinguishable from controls, while all of the 2.0 mg/kg dose group samples were still moderately edematous. These results demonstrate the efficacy of specific behavioral tests to show toxic effects of TET in otherwise asymptomatic animals.

Male Fischer 344 rats were given 14 doses of triethyl tin (TET) subcutaneously over a period of three weeks. Responsiveness to painful stimulation and muscular strength was measured at the end of dosing and after two weeks of recovery. Responsiveness to painful stimuli was elevated by TET in tests requiring a relatively large motor response (tail flick, hot plate) and was associated with concurrent decreases in hindlimb grip strength. Reactivity as measured by an operant titration procedure requiring a nose poke response was not affected by TET. All neurotoxic effects of TET were reversible by two weeks after cessation of dosing. These data suggest that TET does not affect reactivity to pain whenever TET-induced neuromotor deficits are taken into account.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will attempt to correlate the sensitivity of select functional tests with the pathology of tin toxicity and with changes in behavioral responses such as spontaneous motor activity and consummatory behaviors. The results of these studies may lead to the identification and application of highly sensitive biobehavioral assays to biomonitor the chronic effects of environmental neurotoxic agents such as the heavy metals.

PUBLICATIONS

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

Burne, T.A. and Tilson, H.A.: Titration procedure with rats using a nose poke response and tail shock. Pharmacol. Biochem. Behav. 13: 653-656, 1980.

Tilson, H.A. and Burne, T.A.: Effects of triethyl tin on pain reactivity and neuromotor controls of rats. J. Toxicol. Environ. Hlth. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90012-03 LBNT

PERIOD COVERED
October 1, 1980 to January 1, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Developmental Exposure to Monosodium Glutamate on the Neurobehavioral Development of Rats

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

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS
	C. A. Lamartiniere	Senior Staff Fellow	LOFT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Organ Function and Toxicology, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Postnatal administration of large concentrations of monosodium glutamate (MSG) is known to produce lesions in the arcuate nucleus of the hypothalamus. The effects of intermediate doses of MSG given neonatally behavioral and neurological functioning of adults are being studied in this laboratory so as to generate a profile of effects from a known neurotoxicant. Such information will provide a basis for mechanistic studies with environmental neurotoxicants.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: On days 1-5 postpartum male and female Sprague-Dawley derived CD strain pups were dosed s.c. with either L-glutamic acid (MSG) (2-3.5 mg/g), 13% or 0.85% saline and tested at 67 and 102 days of age. At both periods, the body weights of MSG-exposed males were less than the 13% exposed isosomotic controls. MSG-exposed females, however, appeared to be obese compared to their controls at 102 days and exhibited a 50% incidence of tail-automutilation. Exposure to MSG did not affect the startle responsiveness of males or females to an acoustic startle stimulus. The startle responsiveness of females to a tactile air puff stimulus was significantly depressed in amplitude at 67 and 102 days; the response of the males at 67 days of age was also decreased, but the effect was not statistically significant. Fore- and hindlimb grip strength assessments indicated that MSG-exposed females, at 102 days, had greater hindlimb grip strength. Forelimb grip strength was not affected in either sex. Tail flick latencies to a thermal stimulus were significantly elevated at 67 and 102 days of age in both MSG-exposed sexes. Relative to the isosmotic control group, spontaneous motor activity of MSG-exposed animals was found to be consistently lower. Exposure to MSG did not, however, change the responsiveness of either sex to the motor activity stimulating effects of a d-amphetamine challenge (0.3-3 mg/kg). These results indicate that postnatal exposure to MSG produced measurable, long-term behavioral and somatic alterations in females and, to a lesser degree, male rats.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The study of how exposure to environmental agents during the period of sexual differentiation affects neurobehavioral functioning may yield information concerning the neurohumoral and neuroendocrine mechanisms underlying developmental effects.

PUBLICATION

Squibb, R.E., Tilson, H.A., Meyer, O.A., and Lamartiniere, C.A.: Neonatal exposure to monosodium glutamate alters the neurobehavioral performance of adult rats. Neurotoxicology in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90013-03 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurotoxic Effects of Repeated Exposure to Kepone in Rats

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

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS
	J. A. Moore	Deputy Director	NTP	NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	J. A. Rosecrans	Research Pharmacologist	LBNT	NIEHS
	L. L. Uphouse	Psychologist	LBNT	NIEHS

COOPERATING UNITS (if any)

National Toxicology Program

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	0.9	1.9

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Kepone is an insecticide used for the control of fire ants. Several workers were exposed to relatively large amounts of this agent and large quantities are known to have been released in certain areas in the state of Virginia. The purpose of this research is to (1) provide a profile of kepone neurotoxicity in an animal model for comparison with clinical reports of neurotoxicity and (2) attempt to identify some of the neurochemical effects of kepone.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

A. Short-term, dose ranging study (51 days). Male and female albino rats of the Fischer-344 strain weighing approximately 250 g at the start of the study received 0, 10, or 30 ppm kepone administered in the diet for a period of 51 days. Five days after cessation of dosing, the rats were given a battery of neurobehavioral tests. Males receiving 10 and 30 ppm kepone had an exaggerated startle response to an air puff stimulus (172 and 214% of control, respectively) while females were not significantly affected. The proportion of animals emerging into an open field within 100 sec was significantly decreased in female rats receiving 10 and 30 ppm kepone (3/8 for both groups, as compared to 10/11 for controls), but males were not affected. Body weights of the females given 30 ppm kepone were 94% of control which was a significant decrease. No other effect on this measure was noted. Significant effects on fore- and hindlimb grip strength were not observed. Latencies to escape shock in a one-way avoidance task were not affected in rats given kepone, but males given 30 ppm kepone had significantly increased avoidance latencies (mean of 7.2 sec, as compared to 5.5 sec for controls). All the animals were retested for recovery of function at 28 days after cessation of dosing. At that time, the only significant behavioral effects noted were increased startle magnitude in males exposed to 30 ppm kepone (133% of control) and increased retention latencies for males exposed to 10 and 30 ppm kepone (128 and 131% of control, respectively) and for females exposed to 30 ppm kepone (213% of control). These results indicate that repeated exposure to kepone in the diet of rats increased responsiveness to some types of environmental conditions and possibly impaired the acquisition and retention of a simple avoidance task. Kepone neurotoxicity was observed in both sexes in the absence of body weight changes or muscular weakness, and it appeared to dissipate with time following cessation of exposure.

B. Ninty-day chronic dosing. Male rats fed 0-30 ppm of kepone showed no significant changes in body weight, fore- or hindlimb grip strength, negative geotaxis, or tail flick responses. Startle to an air puff was elevated in the 30 ppm group after 30, 60, and 90 days of dosing, while rats at the 10 ppm dose were similarly affected after 90 days of dosing. Rats receiving 10 and 30 ppm had an elevated auditory startle at 30-90 days of dosing. Free operant activity and acquisition of a two-way shuttle box avoidance response were not affected after 90 days of dosing. Recovery of function was observed 30 days after cessation of dosing.

C. Previous work in the Laboratory suggests that the primary neurotoxicological effect of kepone is exaggerated startle and tremor. Experiments are now underway which will study the time course, dose response relationship between acutely administered kepone and these two neurobehavioral functions. Similar studies using a repeated dosing regimen are also planned.

Additional work is planned which will investigate the possible mechanism of action of the kepone-induced neurotoxicity following acute and repeated

dosing. Basically, two hypotheses are under consideration: (1) the neurotoxicity of kepone may be related to anticholinergic activity; attempts will be made to alter tremor/startle alterations using pharmacological tools or blockers and (2) the neurotoxicity of kepone may be related to the estrogenicity of the chemical; tremor/startle will be studied in conjunction with various treatments that systematically vary the level of circulating estrogen.

D. Kepone appears to alter somatosensory receptivity. Studies are planned which will systematically determine the effects of kepone on responsiveness to various environmental stimulation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effects of repeated exposure to environmental contaminants such as kepone are of continuing concern to environmental health scientists. The systematic study of the toxicity of kepone is of concern because of the specific sequela of neurotoxicity observed in humans. Evaluation of substances such as kepone, which clearly affect human health, is clearly within the mandate of the Institute.

PUBLICATIONS

Tilson, H.A. and Mitchell, C.L.: Models for Neurotoxicity. In Hodgson, E., Bend, J.R., and Philpot, R.M. (Eds.): Reviews in Biochemical Toxicology. New York, Elsevier/North Holland, 1980, pp. 265-294.

Squibb, R.E. and Tilson, H.A.: Neurobehavioral changes in adult Fischer 344 rats exposed to dietary levels of chlordecone (Kepone®): A 90 Day of Study. Toxicol. Appl. Pharmacol. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90019-02 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Toxic Agents: Effects on Neuropeptides

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

PI:	J. S. Hong	Pharmacologist	LBNT	NIEHS
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	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	C. R. Hung	Visiting Fellow	LBNT	NIEHS
	J. A. Rosecrans	Research Pharmacologist	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	L. L. Uphouse	Psychologist	LBNT	NIEHS
	W. E. Wilson	Research Chemist	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry 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 (200 words or less - underline keywords)

The neuropeptide profile is being examined in animals treated with various neurotoxicants. Single injection of acrylamide (100 mg/kg; p.o. killed 24 hr after) failed to alter the brain [Met^5]-enkephalin, substance P, or neurotensin levels. However, 10 daily injections of acrylamide (20 mg/kg/day) caused a selective increase in substance P content in striatum. This result suggests that substance P may be related to the tremor seen in acrylamide-treated rats. Repeptive administration of kepone (10 mg/kg/day; p.o. for 10 days) caused a 40% decrease in pituitary [Met^5]-enkephalin in male rats. Since there is a sex difference in pituitary [Met^5]-enkephalin content (female's content is about 60% of males), plus the reported estrogen-like action of kepone, these results tend to suggest that pituitary enkephalin may be under the regulation by gonadosteroid hormones.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The brain neuropeptides content is measured by radioimmunoassay methods by using specific antiserum against the neuropeptide to be measured. To enhance the specificity of radioimmunoassay, peptides will be physically separated by high performance liquid chromatography before they are assayed.

MAJOR FINDINGS AND PROPOSED COURSE: Several lines of evidence suggest that some neuropeptides such as substance P or enkephalins may be important in regulating the motor or psychological functions. In an attempt to obtain further information consistent with this notion, we studied the effects of acrylamide administration on neuropeptide levels in rat brains. Acrylamide is known to produce neuropathy both in humans and experimental animals. After 10 daily oral administrations of acrylamide (20 mg/kg/day) the rats exhibit some movement disorders such as tremor or ataxia. The treatment caused a selective increase in substance P content in striatum, but not in frontal cortex hypothalamus or brain stem. The [Met^5]-enkephalin and neurotensin levels were not altered after such treatment. The selective change of striatal substance P content elicited after acrylamide administration further suggest the role of this peptide in regulating the striato-nigral neuronal functions.

Recently, we have found that the pituitary [Met^5]-enkephalin content of rats exhibits sex difference (female's content is 60% of males). Castration of male rats either in neonatal or adult age decreases the pituitary ME content by 30% suggesting that this peptide may be under the regulation by the gonadosteroid hormones. Because of the reported estrogen-like activity of kepone in reproductive systems, we also tested the possibility that kepone may also exert similar actions on other neuroendocrine glands, such as pituitary. Administration of kepone either neonatally or at adult age decreased the rat pituitary [Met^5]-enkephalin content by 30%. This result suggests that kepone administration may "feminize" the pituitary [Met^5]-enkephalin content. The hypothalamic [Met^5]-enkephalin content and serum prolactin level were also decreased after kepone treatment. Since prolactin release is under the tonic inhibitory influence by dopamine neurons and in turn dopamine neurons is inhibited by endorphins, the decrease in [Met^5]-enkephalin content may be related to the decrease in serum prolactin level.

To extend this line of research, we plan to study the neurotoxicity of manganese. The manganese poisoned miners exhibited early psychiatric disorders manifested by hallucination, confusion, and delirium, which were followed by more stationary neurological disorders which resemble Parkinsonism. Because of the dual effects exerted by manganese, this heavy metal may provide a good chance to study the role of neuropeptide in regulating the motor and psychological functions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The screening of brain extracts and serum for abnormality of neuroendocrine and neuropeptide content is likely to offer a sensitive and original index of hormonal imbalances caused by toxic agents. Many toxicants such as kepone and

monosodium glutamate appear to cause hormonal imbalances and we are testing whether such a series of radioimmunoassays offer potential as an index of neurotoxicity.

Evaluation of which hormonal or peptide compounds are most easily altered in level by toxicants may lead to understanding of which features of brain metabolism are especially vulnerable to chemical derangement.

PUBLICATIONS

Hong, J.S. and Schmid, R.: Intrahippocampal distribution of [Met^5]-enkephalin. Brain Res. 205: 415-418, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90021-02 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Developmental Neurotoxicity of Triethyl Tin and Related Compounds

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

PI: H. A. Tilson Head, Behavioral Toxicology Workgroup LBNT NIEHS
Other: C. L. Mitchell Laboratory Chief LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Triethyl tin (TET) is an organo metal representative of a class of chemicals that produce vacuolization and splitting of myelin. The following research concerned the effects of TET on the developing rat pup. Rats were dosed on postnatal day 5 and neurobehavioral functioning from 21-150 days of age was assessed using neurobehavioral, psychopharmacological, and neurochemical procedures. Postnatal TET appeared to have long lasting effects on behavior and altered the responsiveness of rats of both sexes to apomorphine, a dopamine receptor agonist.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Offspring of time-bred Fischer rats were randomly fostered to dams resulting in four pups of each sex per litter. On day 5 post partum, the pups received a s.c. injection of 15% ethanol vehicle, 1.5 or 3 mg/kg TET in a volume of 100 µl/100 g. The following results have been obtained:

1. TET did not affect body weights as measured on days 5, 7, 14, 21, 28, 60, and 90 days of age.

2. TET had no effects on negative geotaxis or grip strength at 21-90 days of age.

3. Startle responsiveness was decreased with the most prevalent effect noted at 60 days of age.

4. TET increased spontaneous motor activity at 21-60 days of age.

5. Two-way shuttle box acquisition was impaired in females at 60, but not 90 days of age. Males were not affected.

6. Latencies to emerge into a novel environment were elevated in both sexes at 60 and 90 days of age.

7. Ovaries of females exposed neonatally to TET were decreased in weight at 90 days of age.

These data indicate that a single postnatal exposure to TET can produce long-lasting neurobehavioral alterations in the absence of effects on body weight.

Subsequent studies with rats exposed neonatally to 3 mg/kg of TET have found:

1. An exaggerated response to the hypermotility inducing effects of 1 mg/kg of apomorphine.

2. An exaggerated response to the stereotypy produced by 1 mg/kg of apomorphine.

3. Female rats exposed neonatally to TET have lower baseline operant response rates on a variable interval schedule of reinforcement; males have an elevated baseline response rate.

4. TET exposure does not influence the responsiveness of rats to the rate suppressant effects of apomorphine (0.0312-0.25 mg/kg) or areocoline (0.3-2.4 mg/kg).

5. Postnatal exposure to TET did not affect significantly ^{3}H-spiroperidol binding in the striatum or QNB binding in the striatum or hippocampus.

These data suggest that postnatal exposure to TET increases the sensitivity of the postsynaptic dopamine receptor but does not appear to affect the affinity or binding characteristics of the receptor.

Postnatal exposure to TET was also found to alter the responsiveness of exposed animals in a two-choice visual discrimination task. TET-exposed rats responding as the visual discrimination task showed a greater loss of stimulus control during reversal of the contingencies than controls.

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

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Triethyl tin and other compounds known to produce demyelination of central and peripheral neurons are widespread in the environment. The experiments described above have provided some insight as to the types of behavioral tests that are appropriate for detecting developmental neurotoxicity and have led to the elucidation of how long-term behavioral deficits might be caused by exposure to neurotoxicants.

PUBLICATIONS

Harry, G.J. and Tilson, H.A.: The effects of postpartum exposure to triethyl tin on the neurobehavioral functioning of rats. Neurotoxicology in press.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 90023-02 LBNT

PERIOD COVERED
October 1, 1980 to January 1, 1981

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Effects of Benzene Exposure During Postnatal Development

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

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Benzene is a cyclic aromatic hydrocarbon used as a solvent and reactant in a variety of laboratory, commercial, and industrial applications. Large quantities of benzene are also emitted into the environment in motor vehicle emissions, crude oil spills, and emissions from the production of coke from coal. Although the toxicological effects of benzene have been studied in adult animals, the effects on the developing organism have not been extensively determined. The purpose of the following experiment is to: (1) identify methods sensitive to the neurobehavioral effects of developmental exposure to benzene and (2) attempt to characterize any long-term neurobehavioral deficits following exposure of rats to benzene during the postnatal period of development.

PHS-6040
(Rev. 2-81)

PROJECT DESRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The litters from six time-bred Fischer-344 rats were used as subjects. At birth, litters were culled to four males and four females each and the pups were randomly assigned to six foster mothers. On days 9, 11, and 13, the pups from three litters were dosed s.c. with 550 mg/kg of benzene, while the remaining pups received corn oil (vehicle). Benzene had no effect on body weights at anytime during the study. Neurobehavioral toxicity was assessed in a battery of tests at 45, 60, and 100 days of age. Benzene did not appear to affect fore- and hindlimb grip strength, negative geotaxis, or startle responsiveness (sound and air puff stimuli). At 100-130 days, motor activity was measured in an automex during 30 minute sessions; benzene-exposed rats had higher activity levels than controls. When challenged with various doses of d-amphetamine (0.3-3 mg/kg), it was found that the benzene-exposed animals were less sensitive to the motor stimulating effects of d-amphetamine. At 160 days of age, the animals were assessed for exploratory activity (rearing, nose-poking, lever touching) in an operant chamber. Computer analysis of these activities indicated that benzene-exposed rats emitted fewer overall responses in the last third of a 30 min test session. These data indicate that postnatal benzene exposure can produce significant alterations in the activity of rats when tested during adulthood and the type of effect (increase or decrease) depends on the procedure used. Changes in the sensitivity of benzene-exposed rats to d-amphetamine suggest alterations in catecholaminergic function.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developing organism is very sensitive to the presence of many environmental factors. It is within the purview of the missions, goals, and objectives of the Institute and our program to develop methods capable of detecting long-term perturbations in central nervous system functioning following developmental exposure to chemicals related to the energy and petrochemical industries.

PUBLICATIONS

Tilson, H.A., Squibb, R.E., Meyer, O.A., and Sparber, S.B.: Postnatal exposure to benzene alters the neurobehavioral functioning of rats when tested during adulthood. Neurobehav. Toxicol. 2: 101-106, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90024-02 LBNT

PERIOD COVERED
January 1, 1981 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Developmental Neurotoxicity of Kepone in Rats

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

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT	NIEHS
	J. A. Moore	Deputy Director	NTP	NIEHS
	C. F. Mactutus	Staff Fellow	LBNT	NIEHS
	J. A. Rosecrans	Research Pharmacologist	LBNT	NIEHS

COOPERATING UNITS (if any)

Research Resources Program, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Kepone is an insecticide used to control fire ants. Few studies have been reported concerning the developmental neurotoxicity of this chemical. The purpose of the following research is to (1) determine methods suitable for the study of developmental toxicity produced by exposure to environmental neurotoxicants, (2) characterize the developmental neurotoxicity of kepone in rats, and (3) attempt to identify some of the effects of developmental exposure on neurotransmitter function.

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE:

A. Fischer-344 rats were exposed throughout gestation and the first 12 days of lactation to 1 or 6 ppm chlordecone via the maternal diet. Exposure to chlordecone had no effect on maternal weight gains after gestation nor did it affect birth weights, litter sizes, or sex ratio of the litters. Offspring tested at 30 and 100 days of age for behavioral performance showed no significant changes in fore- and hindlimb grip strength, spontaneous motor activity, startle responsiveness, or tail flick latencies to thermal stimulation. Negative geotaxis latencies were not affected at 30 days of age but were increased in males at 100 days of age. Male rats exposed to chlordecone were found to be hypersensitive to the motility increasing effects of 1 mg/kg of apomorphine, which is a dopamine receptor agonist. In another pharmacological challenge with 2 mg/kg of d-amphetamine, a presynaptic releaser of dopamine and norepinephrine, the motility response tended to be exaggerated in chlordecone-exposed rats, but the effect was not statistically significant. These results suggest that fetal and neonatal exposure to chlordecone produces long-term alterations in the postsynaptic function of dopamine receptors. Additional studies to elucidate the behavioral responsiveness of the kepone-exposed animals are planned.

B. On postnatal day (PND) 3, litters of Fischer-344 rats were culled to 4 pups/sex. On PND 4, the pups received s.c. injection of distilled water, dimethylsulfoxide (DMSO), or 0.2 or 1 mg/pup kepone dissolved in DMSO in a volume of 20 µl/pup. As reported by Gellert (Env. Res., 16: 131, 1978), kepone induced precocious vaginal opening. When tested in a battery of neurobehavioral tests at PND 30 and 100, no treatment differences were observed. Rats of both sexes receiving 1 mg of kepone or DMSO on PND 4 were divided into two groups for additional behavioral testing after PND 100. Those in Group A were trained to touch a lever for food reinforcement on a VI 15 sec schedule. Rate of free operant activity habituation differed in kepone-treated rats on day 1 of training, but no differences were observed in acquisition of the task. Following establishment of baseline responding, kepone-treated females showed a significantly lower baseline rate than controls. Males did not differ from controls. Animals in Group B were trained in a discrete trial, two-choice discrimination procedure to make a nose poke response for food. No differences between treatment groups were observed during discrimination training. Once the rats had learned the task (>80% correct trials), contingencies were reversed for two weeks. Kepone-exposed rats of both sexes made significantly fewer intertrial responses than controls. These data indicate that a single postnatal injection of kepone can produce subtle alterations in behavior in the absence of body weight changes and neurological deficits. Additional studies to determine the neonatal effects of kepone on learning are planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developing organism is highly sensitive to many environmental factors. Techniques are needed which can detect and characterize neurobehavioral toxicity following developmental exposure. The experiments described in this report are

designed to assist in the standardization of such tests. In addition, kepone is an environmentally prevalent agent whose developmental neurotoxicity is not well studied. The present investigation will provide some insight as to the mechanisms by which kepone might affect the developing organism.

PUBLICATION

Squibb, R.E. and Tilson, H.A.: Effects of gestation and perinatal exposure to chlordecone (Kepone®) on the neurobehavioral development of Fischer-344 rats. Neurotoxicology in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90025-02 LBNT

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of the Japanese Quail as an Animal Model in Neurobehavioral Toxicology

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

PI:	P. A. Cabe	Senior Staff Fellow	LBNT	NIEHS
Other:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	C. L. Mitchell	Laboratory Chief	LBNT	NIEHS

COOPERATING UNITS (if any)

Poultry Science Department, North Carolina State University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Generalizability of neurobehavioral toxicology results benefits from studies on multiple species. Some species may provide unique behavioral (or other) capabilities for testing particular effects. Japanese quail (Coturnix coturnix japonica) have practical advantages for neurobehavioral testing (small size, hardiness, highly developed auditory and visual systems, well defined social behaviors). Development of a standardized battery of neurobehavioral screening tests will be undertaken to assess the usefulness of Japanese quail as a model for neurotoxicant effects. Validation of methods will entail the demonstration of sensitivity to drug and/or toxicant treatment. Sensitivity to lethal effects of acrylamide has been tested, as a possible model neurotoxicant. Several reflex measures (flip test, wing extension) have been developed and preliminary effects of some drugs have been tested.

PROJECT DESCRIPTION

METHODS EMPLOYED: Neurobehavioral toxicity screening methods for use with rodents have been well documented. It is proposed that an analogous screening battery be developed for use with Japanese quail. Primary tests of visual function, auditory function, pain sensitivity, and vestibular function are planned, as well as measures of spontaneous activity, neuromuscular function, and arousal/reactivity. In addition, routine body weight and body temperature readings will be collected.

Secondary tests to be considered following primary screening will include performance on operant schedules of reinforcement and measures of learning and memory.

The validation scheme will have several parts: (a) identification of candidate procedures and development of standard protocols for each measure or test; (b) collection of normative data and reliability estimation; (c) determination of dose-effect relations for pharmacological agents; (d) determination of dose-effect relations for toxicants having relatively predictable effects; and (e) test for sensitivity to the effects of unknown toxicants.

PROPOSED COURSE: The proposed course will follow the points listed above as components of the validation scheme. Measures for vehicle equipment and/or procedures already exist (spontaneous activity or righting reflex, for example) will be examined first, while methods for other categories of functions are identified and procedural protocols written.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Comparison of results from a range of species is necessary to reduce the possibility of overlooking an effect due to a species idiosyncrasy. Convergent or parallel results from several species supports extrapolation of results to man, particularly where its species do not have a dose phylogenetic kinship. The major goal of the Institute is the prediction of human disease; expanding the ability to show cross-species generality of neurobehavioral effects of agents should increase our ability to predict neurobehavioral consequences of human exposure to neurotoxicant agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90028-02 LBNT

PERIOD COVERED
September 30, 1980 to March 31, 1981

TITLE OF PROJECT (80 characters or less)

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

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

PI:	C. L. Mitchell	Laboratory Chief	LBNT NIEHS
Other:	R. E. Squibb	Senior Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.15	0.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPIION

METHODS EMPLOYED: Thirty-two male, 90 day old Fischer-344 rats were dosed p.o. twice a week with 0, 20, 40, or 80 mg/kg 2,4-D suspended in a corn oil vehicle. Over a five week dosing period they were given a total of 10 doses. Sensorimotor functioning of all animals was assessed using a battery of neurobehavioral tests. These tests were performed once prior to dosing, after each week of dosing and at 30 and 60 days postdosing.

MAJOR FINDINGS AND PROPOSED COURSE: Prolonged exposure to orally administered 2,4-D over a five week dosing period resulted in temporary loss of body weight at high doses. Fore- and hindlimb grip strength generally increased with continuing exposure to 2,4-D. These effects did not persist beyond 60 days postdosing. Exposure to 2,4-D did not alter the mangitude of startle responsiveness to either acoustic or air puff stimuli and did not affect the performance of exposed animals in a test for negative geotaxis. Foot splay enhancement in 2,4-D exposed animals did not occur until 30 and 60 days postdosing; this effect was statistically significant in animals exposed to the higher dose of 2,4-D. Periodic visual observations during and after the time course of dosing indicated that all 2,4-D exposed animals were in good health with no obvious signs of neurotoxicity. Future studies will be designed to systematically assess the time course of neurotoxic effects in animals exposed longer and to lower levels of 2,4-D.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 2,4-D is widely used in our environment as a potent herbicide. The effects of prolonged exposure to 2,4-D on neurobehavioral functioning and its precise effects on the nervous system needs to be systematically investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90029-01 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Chlordecone on the Central Nervous System

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

PI:	L. L. Uphouse	Psychologist	LBNT	NIEHS
Other:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS
	P. K. Seth	Visiting Scientist	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	W. E. Wilson	Research Chemist	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	2.1	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Chlordecone became widely recognized as a neurotoxicant when, in 1975, several individuals received high level acute exposure in a factory in Hopewell, Virginia. Symptoms of exposure include tremors, hyperreactivity, and reproductive malfunction. Since tremor and hyperreactivity are indicative of functional changes in the CNS, we are investigating the effects of chlordecone poisoning on neurotransmitter receptor binding site density, levels, and turnover; and levels of neuroactive peptides are being examined. Pituitary gonadotrophins and steroid hormone levels are being measured to assess the contribution of neuroendocrine changes to the neurotoxicity of chlordecone. In addition, the interaction of chlordecone with the estrogen receptor is being measured and the effect of estrogen on symptoms of chlordecone neurotoxicity are being examined.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Receptor binding density is measured by binding of radioactive ligands to membranes prepared from striatal, hypothalamic, hippocampal, cortical, and cerebellar tissue. Levels of amines and turnover are measured by HPLC. Peptide content and serum levels of gonadotrophins are assessed by radioimmunoassay and levels of gonadal hormones are measured by RIA. Interaction with the estrogen receptor is evaluated by binding of radioactive estradiol or chlordecone to cytosol preparations and precipitation of the 8S receptor by protamine sulfate.

MAJOR FINDINGS AND PROPOSED COURSE: In the adult male rat, 90 days of exposure to chlordecone decreases ^{3}H-spiroperidol and ^{3}H-QNB binding to striatal membranes and ^{3}H-muscimol binding to cerebellar membranes. Ten days cumulative exposure decreases ^{3}H-spiroperidol binding to forebrain membranes and decreases enkephalin content in the pituitary. Serum LH and testosterone levels are reduced.

Gestational/lactational exposure to chlordecone produces an increase in ^{3}H-spiroperidol binding to striatal membranes. The effect is sex-specific, occurring only in males, and is transient, disappearing in adulthood. When exposure is limited to a single day (4 days of age) during the period of sexual differentiation, chlordecone (in DMSO) produces a transient increase in striatal spiroperidol binding in males and a decrease in forebrain spiroperidol hypothalamic ergocryptine binding in females. Most differences in receptor density disappear in adulthood. However, the consequences of neonatal exposure are still present as a decreased enkephalin content in the pituitary. Evidence of hypothalamic-pituitary disruption are seen as a decreased prolactin level in treated male and female rats and in the development of acylic, persistent vaginal estrus in females.

Chlordecone interacts with the CNS 8S estrogen receptor as judged by its ability to compete with 17-β-estradiol *in vitro*. *In vivo*, estrogen potentiates the development of tremors after chlordecone exposure.

Future work in this area will include:

1. Detailed studies of the time course and dose response curve for the development of tremors.

2. Detailed biochemical analyses (over time and across dose) of the effects of chlordecone on neurotransmitter binding, turnover, peptide content and pituitary secretion.

3. Further evaluation of the affinity of chlordecone for the neural estradiol receptor and the regional distribution of chlordecone binding.

4. Study of chlordecone's interaction with other neural steroid receptors.

5. Evaluation of the role of the pituitary in chlordecone's effect on the nervous system and of estrogen's potentiation of the neurotoxicity.

6. Further studies on the consequence of chlordecone's exposure during sexual differentiation.

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

PUBLICATIONS

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90030-01 LBNT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Factors Influencing the Effects of Toxicants on Neurotransmitter Chemistry

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

PI:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
Other:	S. F. Ali	Visiting Fellow	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS
	C. R. Hung	Visiting Fellow	LBNT	NIEHS
	S. B. Por	Visiting Fellow	LBNT	NIEHS
	P. K. Seth	Visiting Scientist	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	L. L. Uphouse	Psychologist	LBNT	NIEHS
	W. E. Wilson	Research Chemist	LBNT	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.3	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Methods have been developed to allow the evaluation of neurotoxicity by testing for changes in neurotransmitter-related biochemistry in treated animals. A series of transmitter receptor sites can now be assayed; as can monoamines and their catabolic products by high performance liquid chromatography. Other techniques include radioimmunoassay of circulating endocrine hormones and of neuropeptides. By such approaches, the following factors have been identified as potential contaminants or modulators of the neurochemical response: (a) Prior handling experience, (b) sex of animal, (c) the vehicle in which the toxic agent is administered, (d) the age of the animal, and (e) the time of day at which the animal is treated or killed. In addition, asymmetric distribution of several biochemical parameters has been found between left and right brain regions. Using acrylamide as a model compound, evidence suggests that toxicants can influence cerebral chemistry (a) directly, (b) by way of the endocrine system, and (c) after conversion by hepatic enzymes to a toxic catabolite. A relation between changes in the dopamine system and altered behavior after a pharmacological agent was suggested. Studies on manganese-treated rats show receptor binding assays may be used to detect selective damage to a specific neuronal circuit.

PHS-6040
(Rev. 2-81)

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.

Levels of monoamines and their catabolic products are determined by high performance liquid chromatography. Membrane are prepared from control animals or from rats treated with toxicants. Some studies involve the addition of toxic agents to the incubation medium in order to determine whether effects on transmitter binding are direct or secondary.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Development of Techniques

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

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

   High affinity binding methods have been developed and appropriately characterized to as to permit assay of the following receptor sites: dopamine, α-adrenergic, β-adrenergic, serotonin, glycine, γ-aminobutyric acid, muscarinic acetylcholine, benzodiazepine, opiate.

   This work has led to the identification of a strychnine bindng site in the retinal pigment epithelium of several species. The endogenous ligand and role of this receptor is not presently understood.

2. Environmental Factors

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

   a. Effect on handling or familiarization of animals to humans upon endocrine and receptor vectors.

   b. Circadian fluctuations of receptor content of several brain regions.

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

d) Developmental neurochemical changes which may modify susceptibility to toxic agents.

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

f) The finding that several neurotransmitter binding sites are asymmetrically distributed within left and right brain halves presents a complication relevant to experiments involving unilateral lesions such as that described below.

g) The differential susceptibility of male and female animals to neurochemical changes induced by toxicants may be related to the endocrine system interference of some agents such as chlordecone (see also Z01 ES 90029-01 LBNT).

3. Studies on Representative Toxic Agents

a) Manganese

Animals dosed with manganese daily for two weeks showed a selective increase in the striatal binding of a dopaminergic antagonist (spiroperidol). The binding of other radiolabeled ligands selective for other transmitter species was unchanged in several brain regions. This change was found at a level of dosing that did not appear to affect the general health of animals as judged by body weight or gross behavior. Furthermore, levels of dopamine and its catabolite DOPAC were unchanged by this dosing schedule. Since manganese is known to be, especially harmful to dopamine neurons, the binding data obtained is consistent with the results of others, in that it is suggestive of a supersensitive response.

b) Acrylamide

The striatal dopamine receptor has been found to be elevated in adult male rats, dosed once or ten times with acrylamide. This change is reversed a week after cessation of dosing. This effect is specific in that other neurotransmitter receptors are unaltered by exposure to the lower doses of acrylamide. When animals are treated with acrylamide at the prenatal or neonatal stage, the development of the dopamine system appears to be retarded as evaluated by ^{3}H-spiroperidol binding. Thus, acrylamide has opposite effects on the dopamine receptor in the immature and mature animal. Experiments involving the use of SKF-525-A, an inhibitor of inducable hepatic mixed function oxidases, suggest that the active agent causing effects on the dopaminergic system may be a metabolite of acrylamide rather than acrylamide directly. Behavioral studies utilizing a pharmacological challenge by assay of apomorphine-induced stereotypy confirm the abnormality of dopaminergic mechanisms in treated rats. Destruction of postsynaptic cell bodies in the striatum by unilateral injections of kainic acid suggests that

acrylamide-induced receptor changes are largely postsynaptic. In vitro incubation of acrylamide with striatal preparations does not alter receptor binding properties. This suggests that receptor changes are secondary to altered neuronal metabolism or activity level. Since acrylamide appears to modify levels of circulating prolactin, it is not clear whether the endocrine changes effected by this chemical precede or result from its direct effects on brain biochemistry.

c) Other Neurotoxic Agents (see also Z01 ES 90029-01 LBNT)

The long-term effects of polychlorinated biphenyls (PCBs) upon behavior of mice one year after gestational exposure namely hyperactivity and a spinning syndrome has been in part related to alterations in striatal dopamine levels and dopamine receptor content. These compounds are thus able to effect permanent changes in neuronal circuitry when given at an early stage of neural maturation.

The use of the high affinity binding assay has led to the identification of a specific metal binding site within molluscan renal calcareous concretions. By this means, toxic metals could be accumulated in polluted waters and enter the human food chain.

The direct effects of several heavy metal compounds upon a variety of receptor systems have also been studied. While lead acetate does not inhibit transmitter binding at 10^{-4}M, a hundred-fold lower concentrations of tri-n-butyl acetate inhibit cholinergic and catecholamine binding. On the other hand, mercuric chloride was more effective in blocking these two receptor sites than was the organic methylmercuric chlordie. This demonstrates that the chemical form of the metal may drastically alter its capacity to modulate ligand-receptor interactions. The polarity of a compound and its ability to interact with sulphydryl groups may be key determinants even in the absence of the blood-brain barrier. The widely varying responses of different receptors to a given compound suggest that no universal peptide sequence exists in all receptors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A major feature of this work is the demonstration of the feasibility of using altered cerebral receptor binding mechanisms as a means of detecting neurotoxicity. We have demonstrated that relatively low doses and brief exposures to several compounds can change binding characteristics. In some cases these changes are specific for a certain transmitter species, thus offering clues as to the neuronal systems that are especially prone to toxic disruption. Both reversible and permanent changes have been found. Ontogenic studies have demonstrated that interference with neural development can have consequences that can be detected at much later times in the mature animals. In addition, we have found that the effects of a toxic agent upon maturation can be in an opposite direction as effects detected in the adult.

The transmitter binding screen that we are developing allows a rapid and objective evaluation to be made of the effects of a deleterious agent upon a series of neuronal pathways. In addition, such modulations have been correlated with behavioral changes. The relevance of such correlations is demonstrated by the use of pharmacological challenges on exposed animals.

PUBLICATIONS

Bondy, S.C. and Agrawal, A.K.: The inhibition of cerebral high affinity receptor sites by lead and mercury compounds. Arch. Toxicol. 46: 249-256, 1980.

Damstra, T.E. and Bondy, S.C.: The Current Status and the Future of Biochemical Assays for Assessing Neurotoxicity. In Spencer, P.S. and Schaumburg, H.H. (Eds.): Experimental and Clinical Neurotoxicology Baltimore, Maryland, The Williams and Wilkins Company, 1980, pp. 820-833.

Bondy, S.C. and Harrington, M.D.: The Preferential High Affinity Binding of the Biological Forms of Amino Acid and Hexose Stereoisomers to Bentonite. In Walker, D. (Ed.): Origin of Optical Activity in Nature. Amsterdam, Elsevier Press, 1980, pp. 141-150.

Bondy, S.C.: Rapid Screening of Neurotoxic Agents by In Vivo and In Vitro Means. In Gryder, R.M. and Frankos, V.H. (Eds.): Effects of Food and Drugs on the Development and Function of the Nervous System: Methods for Predicting Toxicity. U.S. Government Printing Office, 1980, pp. 133-143.

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Damstra, T.E. and Bondy, S.C.: Neurochemical Approaches to the Detection of Neurotoxicity. Target Organ Monographs. New York, Raven Press, in press.

Bondy, S.C.: Neurotransmitter Binding Interactions As a Screen for Neurotoxicity. In Vernadakis, A. and Prasad, K.N. (Eds.): Mechanisms of Neurotoxic Substances. New York, Raven Press, in press.

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

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

Uphouse, L.L. and Bondy, S.C.: The maturation of cortical serotonin binding sites. Develop. Brain Res. in press.

Carmichael, N.G. and Bondy, S.C.: The identification of a specific metal binding site within molluscan renal calcareous concretions. Experientia in press.

Por, S.B. and Bondy, S.C.: Regional circadian variations of acetylcholine muscarinic receptors in the rat brain. J. Neurosci. Res. in press.

Uphouse, L.L., McLean, S.M., and Russell, M.L.: Stability of CNS binding sites under various conditions. Neurotoxicology in press.

STANFORD RESEARCH INSTITUTE - Menlo Park, California 94025
(NO1-ES-8-2154)

TITLE: Test Battery for the Neurobehavioral Assessment of Potential Neurotoxins

CONTRACTOR'S PROJECT DIRECTOR: Gordon T. Pryor, Ph.D.

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

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $109,115

PROJECT DESCRIPTION

OBJECTIVES: The general objectives of the research are to (1) determine the steps necessary for selecting doses to be used in studies involving repeated administration of neurotoxic chemicals and comparison standards in low concentrations; (2) determine the ability of each of a set of neurobehavioral procedures, selected by NIEHS, to detect the presence of an expected neurotoxic effect and determine the capability of each test to provide a negative reading when an effect is not expected; and (3) generate a profile of effects for each substance so that tests assumed to measure the same or similar functions can be compared for relative sensitivity (e.g., cumulative dose required to produce a significant effect).

METHODS EMPLOYED: Standardization and validation of neurobehavioral tests in rats shall be accomplished by evaluating the effects of the following chemicals: tetraethyl tin, methyl mercury, sodium salicylate, d-amphetamine, acrylamide, arsenic, lead acetate, kepone, and triethyl lead.

The subjects are male albino rats of the Fischer-344 strain weighing approximately 200 g at the beginning of the study. The animals are housed individually in stainless steel cages in quarters having a constant light-dark cycle (light, 7 a.m. to 7 p.m.), temperature (21 $\pm$ 2°C), and relative humidity (50 $\pm$ 10%).

Each substance is being evaluated in three steps:

(1) The oral dose required to kill one-half of the subjects tested shall be determined (LD50). Mortality will be observed over a seven day period in at least five treatment groups.

(2) After the LD50 has been determined, a cumulative toxic dose (CTD) shall be ascertained in a 28-day subacute toxicity study involving four groups of ten rats per group. Subjects in the three treatment groups will receive 1/2, 1/4, and 1/16 of the oral LD50 five days a week for four weeks. Those rats in the control group shall receive the vehicle. Daily measures of body weights and mortality shall be used to establish a CTD. The CTD is defined as the dose producing $\leq$ 20% mortality over 28 days of dosing.

(3) Once a CTD has been determined in the 28-day dose-ranging study, portions of the CTD (2/3, 1/3, and 1/6) will be given to another set of test animals by gavage five days per week for 15 weeks. The rats in each of the three treatment groups (n=10 per group) and those rats receiving vehicle (n=20 per group) shall be given a battery of tests the week prior to dosing (Predosing Phase), every third week during dose (Dosing Phase), and in the third and sixth weeks after cessation of dosing (Postdosing Phase). The neurobehavioral assessments shall consist of two parts to be given on consecutive days during each week of testing. Tests include various measures of sensory and motor functioning and indicators of learning ability and include body weight, fore- and hindlimb grip strength, negative geotaxis, startle to an air puff and auditory stimulus, spontaneous motor activity, tail flick, rectal temperature, and performance on a discriminated pole climb avoidance task.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Determination of oral LD50 values: In connection with this project, seven day LD50 values have been established for acrylamide [200 (186-215) mg/kg], tetraethyl tin [13.8 (8.3-22.9) mg/kg], arsenic trioxide [31.8 (26.7-37.8) mg/kg], d-amphetamine sulfate [76.2 (69.3-83.8) mg/kg], monosodium salicylate [1.14 (1.07-1.21) g/kg], triethyl lead chloride [13.4 (11.5-15.6) mg/kg], kepone [89.8 (76.8-105.1) mg/kg], methyl mercury [44.8 (40.3-49.8) mg/kg], and lead acetate [260 (206-328) mg/kg].

(2) Determination of CTD (LD20) from 28 days of dosing has been achieved for acrylamide (26.5 mg/kg), tetraethyl tin (1.5 mg/kg), arsenic trioxide (13 mg/kg), kepone (11.2 mg/kg), methyl mercury hydroxide (2.8 mg/kg), lead acetate (10 mg/kg), d-amphetamine sulfate (60 mg/kg), monosodium salicylate (0.55 g/kg), and triethyl lead chloride (1.4 mg/kg).

(3) Based on the CTD from the 28-day dose-ranging study, 15-week chronic dosing studies have been completed. A summary of the profile for each agent can be found in the attached table. What emerges from this research is that the PTB is capable of detecting changes in motor function, i.e., decreases in grip strength and impaired negative geotaxis. The sensory testing component was not very sensitive; if changes in avoidance responding were observed, they were associated with increases in escape responding and tended to occur for all sensory modalities assessed. In many cases, changes in body weights tended to occur simultaneously with neurological effects. The neurotoxic profiles generated by the battery, however, are in accord with the relative neurotoxicity known to exist in the rat: Tetraethyl Tin > Kepone > Acrylamide > Triethyl Lead > Methyl Mercury > Lead Acetate > Arsenic Trioxide > Monosodium Salicylate.

SIGNIFICANCE TO BIOMEDICAL RESEARCH, THE PROGRAM OF THE INSTITUTE AND THE NATIONAL TOXICOLOGY PROGRAM: The Toxic Substances Control Act of 1976 requires that chemicals prevalent in the environment be evaluated for neurotoxicity. These include pharmaceutical, industrial, and agricultural chemicals, and their by-products. Ideally, for such an evaluation a cost-effective battery of simple animal tests would be used, the reliability and validity of which had been demonstrated experimentally for known environmental toxins. However, a test battery that has general acceptance and that has been adequately

validated is not currently available. The present research will help biomedical research in neurotoxicity by yielding a set of standardized tests and providing basic information concerning the neurotoxicity of the compounds being studied. Such information will be valuable for future studies on the mechanisms of action of these environmental agents.

LABORATORY OF BIOCHEMICAL GENETICS

LABORATORY OF BIOCHEMICAL GENETICS
Summary Statement

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

INTRAMURAL RESEARCH PROGRAM

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

In the Mutation Monitoring Group, the mechanism for induction of mutations in mammalian cells in vivo are studied. Several different selection and detection mechanisms are used for recognizing the mutant cells. Drs. Ansari and Malling have developed the technique for detection of mouse sperm which react with monospecific antibodies to rat LDH-X, indicating the presence of point mutations. This is the first time in history that anyone has detected a type of mutation in sperm which is likely to be point mutation. The frequency of this type of increase is almost proportional to the dose of procarbazine given intraperitoneally to mice. Two more compounds, ethylnitrosourea (ENU) and mitomycin-C, were tested in the LDH-X system and were both positive. Mr. Burkhart and Dr. Malling have investigated the derepression of the LDH-X gene in somatic tissue and found the frequency of mouse liver cells which expressed LDH-X increased after treatment with triethylene melamine (TEM) and ENU. Dr. Wright is in the process of producing monoclonal antibodies to LDH-X by fusion of mouse myeloma cells with lymphocytes from mice which have been immunized with LDH-X. Drs. Ansari, Baig, and Malling are utilizing various hemoglobins of the inbred strains of mice, which differ in as many as thirteen amino acids. Monospecific antibodies have been produced to two different mouse hemoglobin types. A reverse plaque assay test is under development using these two antibodies. If development of this technique were successful that would mean that mutations in a single red blood cell could be detected by visual scanning of slides, instead of using cell sorters and scanning microscopes for detection of the mutant cells. Measurement of the frequency of abnormal sperm is used to screen environmental pollutants. Very little is known, however, about the mechanism which results in an abnormal sperm. Mr. Burkhart and Drs. Binkert and Malling have approached this problem in three different ways: (a) by studying the inheritance of sperm abnormality, (b) by studying the induction of sperm abnormality with various mutagens, and (c) by investigating the mechanism of action of gossypol, a male sterilant in humans. The last compound was observed by Drs. Lee and Malling to inhibit LDH-X and malic enzyme specifically among approximately 20 other enzymes tested. LDH-X is a sperm specific enzyme. Therefore, it is important now to establish whether the inhibition of this enzyme is the major cause of the male sterilizing effect. The DNA content per sperm in inbred mouse strains vary very little. Dr. Malling and Mr. Burkhart are studying the variance of the DNA per

sperm in progeny among mutagen-treated animals. A high frequency of individuals with increased variance of the DNA per sperm were found among such individuals. Several genetic events such as translocations were found to increase the variance of the DNA, but this could only account for a fraction of the detected individuals with higher variance.

The work of the Physiological Genetics Group is concerned with the development of methods for detecting germinal mutations and with the consequences of germinal mutations. Dr. Johnson and his colleague, Dr. Lewis, are examining progeny from mutagen-treated parents for germinally transmitted mutational events. The experimental mutagens which are used include procarbazine, methyl methane sulfonate, and ethylnitrosourea. Methods of analysis are electrophoresis, enzyme activity determinations, and other approaches. A large number of various types of mutations have been detected. These include spontaneous and induced alterations. Mutations are to be characterized as to inheritance pattern, as to their physical/chemical basis (e.g., deletions, base-pair substitutions, frameshifts), for their structural and functional effects on the gene products and according to their metabolic and physiological impact. The purpose of the work is to seek more effective procedures to detect germinal mutations while also providing greater understanding of the impact of individual mutations and of increasing mutation rates generally on the mammalian organism. The work is directly concerned with the problem of human genetic health risks caused by environmental mutagens.

TRAINING PROGRAMS

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. At the postdoctoral level, staff scientists include two visiting fellows from two foreign countries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60021-09 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

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

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

PI:	F. M. Johnson	Research Geneticist	LBG	NIEHS

COOPERATING UNITS (if any)
Research Triangle Institute, Life Sciences Group, Research Triangle Park, NC; Industrial Environmental Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics 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 (200 words or less - underline keywords)

Various approaches to mutation detection are being investigated. Parental mice are exposed to mutagens such as ethylnitrosourea, and transmissible alterations in F_1 progeny are examined. Characteristics include variant enzymes detected by electrophoresis and/or change in activity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Induced and spontaneous mutation rate data are being gathered from the mouse as alternative approaches for the detection of germinal mutation and are evaluated. Methods are based on enzyme characteristics such as activity and electrophoretic mobility. Male mice, some of which are mutagen treated, are mated with females and F_1 offspring obtained. Tissue samples are removed surgically from the parental and F_1 animals and subjected to analysis. Suspected mutants are mated to confirm the genetic basis of variants. Spontaneous and chemically induced mutants affecting phosphoglucomutases, peptidases, malic enzyme, isocitrate dehydrogenase, esterase, hemoglobin, and others have been found. Mutations that cause a reduction in activity are apparently induced at a greater rate than those that alter electrophoretic mobility. Enzyme deficiencies in humans comprise a substantial part of the genetic disease burden. The results from mice may be helpful to understanding the impact of mutational damage to man.

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

PUBLICATIONS

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

Johnson, F. M., Chasalow, F., Lewis, S. E., Barnett, L., and Lee, C.-Y.: A null allele at the Mod-1 locus of the house mouse. J. Heredity 72, 134-136, 1981.

Lee, C.-Y., Chasalow, F., Lee, S.-M., Lewis, S. E., and Johnson, F. M.: A null mutation of cytoplasmic malic enzyme in mice. J. Mol. Cell. Biochem. 30, 143-149, 1980.

Lee, C.-Y., Chasalow, F., Lee, S.-M., Lewis, S. E., and Johnson, F. M.: Identification and biochemical analysis of mouse mutants deficient in cytoplasmic malic enzyme. Biochemistry 19, 5098-5103, 1980.

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, in press, 1981.

Johnson, F. M., Chasalow, F., Anderson, G., MacDougal, P., Hendren, R. W., and Lewis, S. E.: A variation in mouse kidney pyruvate kinase determined by a mutant gene on chromosome 9. Genetical Research 37, 123-131, 1981.

Johnson, F. M. and Lewis, S. E.: Mouse spermatogonia exposed to a high multiply fractionated dose at a cancer chemotherapeutic drug. Mutation analysis by electrophoresis. Mutation Res. 81, 197-202, 1981.

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

Johnson, F. M. and Lewis, S. E.: Mutation rate determinations based on electrophoretic analysis of laboratory mice. Mutation Res., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65002-04 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mutation Studies Using Hemoglobin and Monospecific Antibodies Against Hemoglobins

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

PI:	A.A. Ansari	Visiting Scientist	LBG	NIEHS
Other:	H.V. Malling	Laboratory Chief	LBG	NIEHS
	M.A. Baig	Visiting Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The goal of this project is to develop and test systems that could be used to study mutagenesis in mammals suing single cells. Monospecific antibody against mouse hemoglobins has been developed. Techniques have been worked out for making red cell smears and fixing the cells quantitatively for staining.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Fluorescent antibody technique using monospecific antibody against mouse hemoglobins.

MAJOR FINDINGS AND PROPOSED COURSE: A cellular specific locus mutation test is being developed for detecting mutant cells in mammals. The test is based upon the use of specific anti-C57BL/6 mouse hemoglobin antibody that binds s hemoglobin (present in C57BL/6 mouse) and not d hemoglobin (present in DBA/2 mouse). Attempts to purify such antibody from pony and rabbit antisera through cross-absorption were unsuccessful. Immunization of LP mouse with C57BL/6 hemoglobin produced antiserum that reacted with s hemoglobin but not with d hemoglobin. In a fluorescent antibody technique, this antibody was found to label fixed red blood cells from C57BL/6 mice but not from DBA/2 mice. In a mixture of C57BL/6 and DBA/2 red cells, the C57BL/6 cells could be differentiated by their bright fluorescence from the non-fluorescent DBA/2 cells. Reconstruction experiment with artificial mixtures of DBA/2 and C57BL/6 cells showed that s hemoglobin bearing cells can be detected in DBA/2 red cells at frequencies as small as 0.4×10^{-6}. Thus, the system is sensitive enough to detect d → s mutation in DBA/2 mice. Amino acid comparison of the globin chains of s and d hemoglobins shows that our antibody can probably detect mutations leading to a substitution of serine or proline by alanine at β^{20} position and/or a substitution of threonine by alanine at β^{135} position. A plaque assay has also been developed to detect the red cells carrying abnormal or mutant hemoglobins.

Using this monospecific antibody mutation frequency, spontaneous as well as after treatment with several known and unknown mutagens will be determined. This system could also be developed into a screening system for mutagenic activity of chemicals. Monospecific antibodies will also be developed that react with only monkey hemoglobin and not with human hemoglobin. Such antibodies will be used to study mutation from human to monkey type hemoglobin, and the system could be used as a monitoring system for human population.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: One of the major goals of the Laboratory of Biochemical Genetics is to develop and test systems that could be used to screen chemicals and pollutants for their mutagenic and/or carcinogenic action directly in mammals using single cells. Another goal is to develop methods suitable for monitoring human population for any genetic alterations in exposed individuals. This project is aimed at developing a method that would serve both these goals, screening as well as monitoring.

PUBLICATIONS

Ansari, Aftab, A., Baig, Masroor, A., and Malling, Heinrich, V. PURIFICATION OF FLUORESCEIN-LABELED SPECIFIC ANTI-HEMOGLOBIN ANTIBODY USING CROSS-LINKED IMMUNOABSORBENT. Mutation Research 81 (9181) 243-255.

Ansair, Aftab, A., Baig, Masroor, A., and Malling, Heinrich, V. DEVELOPMENT OF IN VIVO SOMATIC MUTATION SYSTEM USING ANTIBODY AGAINST HEMOGLOBIN-PREPARATION AND USE OF AN ANTI-HEMOGLOBIN ANTIBODY FOR IDENTIFYING C57BL/6 RED CELLS IN ARTIFICIAL MIXTURE OF DBA/2 AND C57BL/6 RED CELLS. Journal of Immunological Methods, 42 (1981) 45-51.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65007-04 LBG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of mutation by using Sperm Specific Enzyme LDH-X

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

PI:	A.A. Ansari	Visiting Scientist	LBG	NIEHS
Other:	H.V. Malling	Laboratory Chief	LBG	NIEHS
	M.A. Baig	Visiting Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.9	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project was undertaken to study mutagenesis using monospecific antibodies against the sperm specific enzyme, lactate dehydrogenase-X. A monospecific antibody has been prepared from rabbit antiserum that reacts with rat sperm and not with mouse sperm. Using this antibody in a sandwich fluorescent antibody technique, point mutations in the mouse sperm were detected in mice treated with mutagenic drugs: procarbazine, mitomycin C, and ethylnitrosourea.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

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

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

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

MAJOR FINDINGS:

Besides procarbazine and mitomycin C, the LDH-X anti LDH-X system has been tested with ethylnitrosourea (ENU) which has been shown to be a potent mutagen in spermatogonia. Mice treated with ENU, at a dose of 200 mg/kg body weight, were used in these studies. The frequency of 'fluorescent' sperm in these treated mice was 50-100 times more compared with untreated mice. The 'mutation' could be detected one year after the drug treatment. Also, mice from the same treatment group gave transmissible mutations studied by Dr. Frank Johnson. These findings further add to the validity of the new mutation system.

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

PUBLICATIONS

Ansari, Aftab, A., Baig, Masroor, A., and Malling, Heinrich, V. In vivo germinal mutation detection with "monospecific" antibody against lactate dehydrogenase-X. Proc. Natl. Acad. Sci. 77 (1980) 7352-7356.

Malling, Heinrich, V. Perspectives in Mutagenesis. Environmental Muta. 3 (1981) 103-108.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65009-03 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of DNA Variance in Sperm

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

PI:	J. G. Burkhart	Research Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. Benziger	Bio. Lab. Tech.	LBG	NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to study the induction and effect of DNA variance in male offspring of mice treated with known mutagens and in certain inbred homozygous mouse strains. Some genetic events such as translocations cause abnormal sperm DNA content but there are other events in spermatogenesis that may also result in sperm with abnormal amounts of DNA. The Axiomat scanning microscope is being used to quantitate the DNA fluorescence of single sperm and spermatids. The measured distributions of DNA fluorescence are being evaluated within and between normal mice, those with known translocations, offspring of treated mice, and inbred strains with a proportion of spermatids that undergo total or partial nuclear non-disjunction during spermatogenesis.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fluorescent labels can be used to quantitate DNA by measuring the relative intensity of emitted light upon excitation of the fluorochrome. Mice have been treated with triethylene melamine and F_1 offspring produced. The male offspring are being mated and pregnant females scored for dominant lethality. Fluorescent data from single sperm of F_1 derived from treated and untreated parents are being collected and analyzed. Testes of mice with abnormal sperm DNA distributions or males that produce significant embryonic death will be evaluated by EM microscopy. All F_1 males will also be scored for translocations by cytogenetic analysis.

MAJOR FINDINGS AND PROPOSED COURSE: At present it has been possible to measure DNA distributions of mature sperm in normal DBA/2J mice and in special strains that carry Robertsonian translocations. The variances of the DNA fluorescence distributions in sperm populations have been found to be greater in the translocation carriers in comparison with normal DBA/2J mice. Future studies will be directed towards maximizing the resolution of the system. In addition certain types of sperm from a homozygous mouse strain that does not have any translocation have been found to have very abnormal DNA content. This mechanism for production of these sperm has been defined. Future studies will be directed to understanding the mechanism and significance of abnormal sperm DNA content. Data will be correlated with other test systems such as the detection of double Y bodies in human sperm.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system may significantly reduce the time, cost and animals needed to assess the potential of compounds to induce chromosomal damage. This work will also help in our understanding the mechanisms and significance of abnormal DNA content in sperm.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65011-02 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Derepression of LDH-X in Mouse Hepatocytes and Lymphocytes

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

PI:	J. G. Burkhart	Research Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	J. Benziger	Bio. Lab. Tech.	LBG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to develop methods to detect the rare expression of LDH-X, a sperm specific isozyme; in single hepatocytes and lymphocytes of mice. The methods are to be used to measure possible changes in the frequency of LDH-X derepression that may result from exposure to environmental agents. Immunofluorescent techniques have been developed to detect LDH-X in mouse hepatocytes fixed on microscope slides. The methodology is being expanded to mouse and human lymphocytes in suspension for counting on an activated cell sorter. The frequency of hepatocytes reacting with anti LDH-X has been measured in male and female LBA/2J mice.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary experiments indicate that the frequency of hepatocytes that react with the anti LDH-X immunofluorescent label system is 7.5-10.0 x 10^{-7} in male and female DBA/2J mice. Preliminary experiments with procarbazine and ethylnitrosourea indicate there is an increase in the frequency of hepatocytes reacting with the anti LDH-X antibody after treatment with these mutagens. Future work will expand the system to peripheral lymphocytes and evaluate if the methodology can be used to detect individuals exposed to mutagens. Changes in the frequency of LDH-X expression that result from treatment will be correlated with data from other mammalian test systems to monitor for mutations and carcinogenesis. In addition, methods will be developed to apply automatic techniques to detect derepression events.

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65013-02 LBG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Plaque-Forming Cell Assay for Detection of Mutation in Mouse Hemoglobins

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

PI:	M.A. Baig	Visiting Fellow	LBG	NIEHS
Other:	A.A. Ansari	Visiting Scientist	LBG	NIEHS
	H.V. Malling	Laboratory Chief	LBG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of undertaking this project is to develop a system, employing the technique of reverse plaque assay that could be used to study spontaneous and induced mutations in mammals using readily available single cells; namely, red blood cells. Antibodies against some of the mouse hemoglobin variants have been raised. A unique plaque assay for mouse red blood cells has been developed using these antibodies. Potential use of the RBC plaque assay for detection of mutation in mouse hemoglobins was studied. Using extensively absorbed goat anti-DBA/2 Hb antibody, as low as 4 DBA/2 RBC could be detected in the presence of a large number of C57BL/6 RBC.

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Antisera. Since antibodies against DBA/2 (D2) or C57BL/6 (B6) mouse hemoglobin (Hb) raised either in other strains of mice, in rabbits, or in horse loose their activity after coupling to sheep red blood cells (SRBS), goats were immunized to raise antibodies against the mouse Hbs because goat antibodies do not loose their antigen binding activity after coupling to SRBC.

2. Purification of specific antibodies from the antisera. In order to obtain an antibody preparation could react only with specific Hb variant and not with others, goat anti-D2 Hb antiserum was absorbed repeatedly on cross-linked B6 Hb (heterologous) immunoabsorbent. The specific anti-D2 Hb antibody was then isolated using a column of D2 Hb (homologous) immunoabsorbent.

3. RBC plaque assay. The method of plaque assay involves the lysis of antibody-coupled SRBC (indicator cells) in the presence of antigen and complement. In a total volume of 170 µl, the assay mixture contained 80 µl of 8% fetal calf serum (FCS) in Hank's Balanced Salt Solution, 96 x 10^6 indicator cells (goat anti-mouse Hb coupled) in 25 µl 8% FCS, desired number of test mouse RBC, 15 µl of multispecific rabbit antibody against mouse Hb and mouse RBC ghost and 20 µl of the three times diluted guinea pig complement. The mixture was poured into plaque chambers, sealed with was and incubated at room temperature for about 2 hrs.

MAJOR FINDINGS AND PROPOSED COURSE:

When SRBC coated with gamma globulin fraction of the unabsorbed goat anti-D2 Hb antiserum were used as indicator cells in the plaque assay, both D2 and B6 RBC formed equally good plaques. No plaque formation took place when (a) the antibody coupled SRBC were replaced with uncoupled SRBC, (b) the test mouse RBC were not added, or (c) the complement or the developer was omitted from the assay mixture. Also, the number of plaques formed was found to increase with increase in the number of mouse RBC in the assay mixture. All these observations clearly demonstrate that the plaques in this assay are formed by mouse RBC and not by anything else. When the SRBC coupled with the purified goat anti-D2 Hb antibody were used as indicator cells in the plaque assay, only D2 RBC formed plaques while no plaques were formed with B6 RBC. When a mixture of a few D2 RBC and a large number of B6 RBC was tested for plaque formation, the number of plaques formed was found to be roughly equal to the number of D2 RBC present in the mouse RBC mixture. However, when the ratio of the D2 RBC to B6 RBC in the test mixture was higher than 1:10, no plaque formation could be observed. This probably was due to the fact that the purified goat anti-D2 Hb could still bind the B6 Hb when present in relatively high concentration.

Attempts will be made to prepare high affinity monoclonal antibodies against mouse Hbs using the hybridoma technique. Once prepared, such antibody will be used to coat SRBC to get indicator cells which can be used in the RBC plaque assay to study mutation in mouse Hbs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: When fully developed, the method could be used to identify chemcials that can cause mutation in mammals using readily available samples. Further, this method can also be employed to monitor human population for genetic injuries. These goals are in accordance with the Institute's program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65022-01 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Types of Sperm Anomalies in Males After Treatment with a Mutagen

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

PI:	F. Binkert	Visiting Fellow	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The aim of this project is to elucidate the connection between mutagenic treatment and type of sperm anomalies. At present different types of anomalies are tested for their usefulness in mutagenic screening. Different doses of methyl methanesulfonate (MMS) served so far as inducers.

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: At present a blind evaluation of the coded slides of a large test series is underway. The result will show which parameter substantiates the effect of the chemical. Other chemicals will later prove the significance of the findings.

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65023-01 LBG

PERIOD COVERED

February 1, 1981 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Use of Monoclonal Antibodies to Detect Mutant Forms of Lactate Dehydrogenase-X in Sperm

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

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

COOPERATING UNITS (if any)

Comparative Medicine Branch; Biometry Branch

LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Mutation Monitoring Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Our goal is to measure frequencies of point mutations in sperm from mice treated with mutagens. Our strategy for detecting mutations is based on immunologic differences in a sperm-associated isoenzyme, lactate dehydrogenase-X (LDH-X) existing as isomeric forms immunologically identifiable to each species (i.e., mouse, rat, humans). Dr. Ansari previously reported that normal antibody to rat LDH-X does not react with LDH-X associated with mouse sperm; however, low frequencies of mouse sperm contain LDH-X that reacts with antibody in rat LDH-X. Moreover, mice treated with the mutagen procarbazine generate increased frequencies of sperm that react with antibody to rat-form LDH-X. The increased frequency of mouse sperm expressing rat-form LDH-X increases linearly with increasing doses of procarbazine. We will develop monoclonal antibodies

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65023-01 LBG . . . continued

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

The use of Monoclonal Antibodies to Detect Mutant Forms of Lactate Dehydrogenase-X in Sperm

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

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

which react specifically with amino acid sequences present in rat-form LDH-X but not in mouse form LDH-X. Monoclonal antibodies coupled with fluorescent markers will be utilized whereby mouse sperm cells will be screened and mutants which are identified will be sorted to confirm that variant sperm express a mutant form of LDH-X. Once the tests are validated, studies will be extended to monitor frequencies of mutant forms of LDH-X in sperm from humans with clinical histories of treatment or exposure to mutagens.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenase-X (LDH-X) is an isoenzyme naturally associated with sperm of many species. Sperm from mice, rats, hamsters, and humans will be collected. From each , LDH-X will be purified and used as an immunogen and injected into mice. Our goal is to produce monoclonal antibodies to each form of LDH-X (i.e., mouse, rat, etc.). Normally monoclonal antibodies to rat LDH-X, for example, would not react with LDH-X 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-X have been substituted by other residues to the extent that the variant form of LDH-X appears immunologically as rat-form LDH-X. Thus, the antibody to rat LDH-X binds to the variant or mutant form of LDH-X 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-X. Rat LDH-X antibody, bound to the variant form of LDH-X 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-X antibody fluorescein markers, will be screened for mutant sperm (containing the variant form of LDH-X). 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-X, not only will be detected by this method but a library of information will be stored concerning the frequencies of mutations at LDH-X in various species treated with mutagens.

MAJOR FINDINGS AND PROPOSED COURSE: Rat and mouse LDH-X have been purified from sperm and injected separately as immunogens into mice. Thus far mouse splenocytes have been fused with each of the following mouse myeloma cell lines: SP.O-Ag 14, NS-1, P3x63 Ag8.653, and P3 x 63 Ag8. Techniques also have been developed to culture feeder layers which enhance the yield of arising hybrids. The proposed course of this investigation is to develop systems when hybrids will be recloned to attain a homogeneous hybrid population secreting pure antibody preparations. Solid-phase indirect enzyme immunoassays, utilizing either p-nitrophenyl-β-D-galactside, alkaline phosphatase, or lactoperoxidase will be developed to determine the activity of antibody to LDH-X. Monoclonal antibodies known to be active to a specific isomeric form of LDH-X (rat LDH-X, for example) will be employed to detect frequencies of mutant forms of LDH-X (in either mice, humans, or hamsters, for example) and perhaps to biochemically analyze mutant forms of LDH-X. 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-X associated with sperm.

SIGNIFICANCE TO BIOCHEMICAL 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 fre-

quencies of mutant forms of LDH-X associated with sperm from human patients with a clinical history of treatment with mutagens such as procarbazine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65024-01 LBG

PERIOD COVERED
October 1, 1980, through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Inheritance of Different Sperm Abnormalities in the BALB/c and the PL Mouse Strain

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

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The aim of this project is to elucidate the inheritance of different sperm abnormalities found in the BALB/c and the PL mouse strain. The sperm of the offspring of the F_1 crosses have been evaluated; the backcrosses are in process of being evaluated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: The results of the finished crosses are the following: The F_1 generation of crosses between BALB/c and DBA/2J shows a recessive trait with a very weak penetrance of the mutant character. The offspring of crosses between BALB/c and PL has, in comparison with PL, a reduced rate of abnormal sperm. The rate, however, is higher than among the offspring from crosses between DBA/2J and BALB/c. There is also a clear-cut maternal effect of the PL-strain. Electron microscope studies and additional crosses will give further evidence of the inheritance.

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: 171-183, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65025-01 -LBG

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Sperm Abnormality and Male Sterility Induced by Gossypol

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

PI: M. L. Snell
J. G. Burkhart
Other: H. V. Malling
G. Lee

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to study sperm abnormality and the action mechanism of male sterility induced by gossypol. The progress of using this drug as a male sterilant has been hampered by some of its toxic side effects and the lack of understanding of the action mechanism of induced male sterility. Dr. Gregory Lee and Dr. H. V. Malling have made an earlier observation that out of over 20 enzymes investigated gossypol inhibited lactate dehydrogenase-X (a sperm specific enzyme). They also observed that there are certain breakdown products of gossypol which had a much stronger inhibition of LDH-X. It is uncertain if gossypol itself, the breakdown products, or both are exhibiting inhibitory effects by the same or different mechanisms. During treatment of animals and humans with gossypol the development of the male germinal cells disintegrate. When the treatment terminates most animals and humans regain their fertility indicating that germinal stem cells were intact. The crucial question is therefore: is the inhibition of LDH-X by gossypol the major course of male sterility or are the correlation of the two biological effects only incidental.

PHS-6040
(Rev. 2-81)

Methods Employed and Proposed Course:

Since there are no reports in the literature describing the effect of gossypol on mice, it will be necessary to run several different sets of animal experiments. The ECB has purchased and stored 100 gm of gossypol in aliquots of 500 mg per vial. In most of the animal experimentation we will use the gossypol as it was acquired.

The animals will be administered various concentrations of gossypol by gavage. At regular intervals (14, 28, and 42 days after initiation of treatment) mice will be sampled and the specific activity of LDH will be measured in the liver, heart, muscle, and testis. The activity of LDH-X will be measured in the sperm. Specific activity of μ-Glycero-phosphate dehydrogenase (μGPD) and succinyl dehydrogenase (SD) will also be measured. Sperm abnormalities induced by gossypol will also be observed.

Based on the above observations, doses will be adjusted to the ones which give the highest sterility and lowest toxicity. Most of the same parameters will be measured again. In addition to mating the treated mice for fertility studies and induction of dominant lethals, the specific activity of LDH, LDH-X, SD, and μGPD will be studied along with sperm abnormality. Testis will also be removed and cut on the freezing microtome for histochemical stains for LDH-X and staining with fluorescent monospecific antibodies to LDH-X. These observations are probably the most critical for understanding the link between LDH-X inhibition by gossypol and male sterility. Sperm from untreated animals will be stained histochemically for LDH, μGPD, and SD activities with or without the presence of gossypol. Crude enzyme preparations will be prepared from sperm and the inhibitory effect of gossypol will be studied on these preparations. The following enzymes LDH-1, LDH-5, and LDH-X will be purified from crude fresh tissue homogenate. Classical kinetics of all the fractions prepared by ECB will be carried out on these preparations.

Significance to Biomedical Research and the Program of the Institute.

Gossypol may be an important male antifertility agent in the near future. It not only is in relatively abundant supply but also very economical as a contraceptive. Therefore studies on its side effects, safety and effects of long term use, and mechanism of inhibitory action are of significant importance.

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

LABORATORY OF ENVIRONMENTAL BIOPHYSICS
Summary Statement

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

NONIONIZING RADIATION

Within the nonionizing radiation program, research is being conducted to: develop microwave exposure systems for bioeffects research; develop and test techniques for measuring microwave energy absorption; determine the effect of microwaves on isolated nerve preparations; determine how 2450 MHz microwave radiation interacts with biological systems at all levels; and investigate the peripheral and central receptors mediating effects of microwave radiation on brain activity.

Waveguide systems for exposing cell preparations which allow stirring during exposure have been developed. These systems provide the capability for accurate determination of specific absorption rates (SAR's) and for controlling temperature at any desired level between 10°C and 60°C. The systems operate at 2450 MHz and are capable of providing specific absorption rates from 0 to 100 mW/g using continuous wave radiation and 0 to 30 mW/g using pulse wave radiation. Two of these waveguide systems have been modified so that circular dichroism and fluorescence spectroscopy can be used to determine microwave interaction with biological systems. A specially designed waveguide inserted into a spectropolarimeter allows the continuous recording of optical activity before, during, and after microwave irradiation. The fluorescent probe, 1-anilino-8-naphthalene sulfonate (ANS) was used to monitor the effect of microwave radiation on the binding of calcium to erythrocyte ghost membranes during exposure. An exposure chamber for single animals, which permits the monitoring of physiological parameters during exposure, has also been developed. A circularly polarized waveguide system is under fabrication which will provide the capability for long-term exposure of animals to 918 and 2450 MHz microwaves while being housed and cared for under near normal animal caretaking conditions.

Research into the biological effects of microwaves at the subcellular, cellular, and organ level is an important component of the nonionizing radiation program. Experiments to determine whether pulsed wave microwave radiation at 2450 MHz produces a different effect from continuous wave irradiation on the viability of isolated frog sciatic nerves have been completed. Three sets of experiments were carried out using 10 microsecond wide pulses at 50 pps, with an average SAR of 10 W/kg: (1) asynchronous pulsing wherein the microwave pulse was delivered at varying times in the firing cycle; (2) synchronous pulsing during the peak of

the nerve action potential; and (3) synchronous pulsing during the quiescent period between nerve firings. In all three cases a signficiant decrease in the survival time of the exposed nerves, as compared to their unexposed mates, was seen. However, the magnitude of this effect was essentially the same in all three cases and was also comparable to the effect seen earlier using CW (of equivalent SAR). These results lend further credence to the hypothesis that the microwave effect on nerve vitality is based on an interaction with long term regulatory processes rather than an interference with the action potential firing mechanism.

Other subcellular and cellular biological material was exposed to 2450 MHz microwave radiation. No effects were observed on the in vitro activity of phosphokinase or acetylcholinesterase at SAR's up to 50 mW/g. Cardiac cells obtained from 9 day old quail embryos exhibited an increase in cell membrane permeability to trypan blue at SAR's of 10, 50, and 100 mW/g following 90 minutes of exposure. Cellular damage was noted at an SAR of 100 mW/g. Lysosome fragility was not influenced by microwaves at SAR's up to 100 mW/g for an exposure duration of 90 minutes. The effect of microwave radiation was studied on active secretory cells. Rat peritoneal mast cells were exposed to 2450 MHz microwave radiation at specific absorption rates (SAR) of 8.5 and 42.5 mW/g for periods of up to 3 hrs. Cells were maintained throughout exposure at 37°C. There was no effect on cell viability or spontaneous histamine release. Mast cells exposed to compound 48/80, after prior irradiation or during simultaneous irradiation, secreted histamine in a manner similar to unexposed cells. In addition, mast cells exposed to concanavalin A or the ionophore A23187 during simultaneous irradiation secreted histamine in a manner similar to unexposed cells. Mature turkey spermatocytes were exposed to 2450 MHz microwaves at SAR's of 1, 10, and 50 mW/g. Viability was determined before and after exposure using accepted staining techniques. Before irradiation the viability was 96 percent, and this viability was not affected by any of the SAR's used in these experiments. The release of the soluble enzymes, lactic acid dehydrogenase and glutamic oxalic transaminase by the sperm into the suspending media was also determined. No changes in the release of these enzymes were observed. Proteins of human erthrocyte ghost were exposed to 2.45 GHz microwave radiation, and effects evaluated using circular dichroism spectroscopy. The data showed that high levels of microwave radiation (SAR = 600 mW/g) induced decreases in the α-helical conformation of membrane proteins that may be due to both thermal vibrations and increased strain on the intramolecular hydrogen bonds that maintain secondary structure. Spectrin (band 1 and 2) isolated from the ghosts was more sensitive to microwave irradiation than intact ghosts, and spectrin-depleted vesicles were the least sensitive. The data, therefore, indicate that the α-helical conformation of membrane proteins is altered by high levels of microwave radiation. Erythrocyte ghost membranes were exposed to 2.45 GHz microwave radiation at SAR's from 0-200 mW/g. A fluorescent probe was used to monitor the effect of microwaves on the binding of calcium to the erythrocyte ghosts. The results show that the radiation did not interfere with the binding of physiological concentrations of calcium to erythrocyte ghosts or alter intermolecular distances between intrinsic molecules and bound ANS.

Research to determine the effects of 2450 MHz microwaves on cardiac function in cats with normal hearts or with myocardial ischemic hearts were performed. The hearts were irradiated directly by using surgical methods to open the chest cavity. The exposed hearts were irradiated at an SAR of 30 mW/g for 5 hours and cardiac function was measured throughout the exposure period. Mean arterial

blood pressure, cardiac output, heart rate, plasma and myocardial creatine phosphokinase (CPK), and S-T segment were not influenced by the microwave radiation in either the normal or the myocardial ischemic hearts. Isolated rat atria have been exposed to 2.45 GHz microwaves at SAR's of 2 and 10 mW/g for periods up to 1.5 hours. Beat rate and contractile force were monitored during exposure. Preliminary data indicate that at the two SAR's used there was no overt effect on the rate or force of contraction of isolated atria maintained at either 22°C or 37°C. Whole bodies of adult, male rate have also been exposed to 2.45 GHz to SAR's of 5 and 10 mW/g on the ventral side. Four hours of exposure had no effect on mean arterial blood pressure or heart rate.

A significant effort has been made to determine the teratogenic and developmental effects of 2450 MHz microwave radiation. Pregnant mice were exposed for 8 hours per day for various period of pregnancy to 5, 21, and 30 mW/cm^2. The group exposed to 5 mW/cm^2 were irradiated from day 1 through day 15 of pregnancy. For the 21 and 30 mW/cm^2 exposures, two different groups of animals were irradiated for different portions of pregnancy, days 1-6 and days 6-15. Groups were also exposed to elevated temperatures to simulate thermal stress (30°C for the 21 mW/cm^2 group and 31°C for the 30 mW/cm^2 group). Exposure to power densities of 5 mW/cm^2 (SAR = 5.3 mW/g) did not produce any adverse maternal or embryofetal effects. Exposure to 30 mW/cm^2 (SAR = 32 mW/g) during days 1-6 resulted in a significant decrease in implantation sites per litter and average fetal weight. Exposure to 30 mW/cm^2 during days 6-15 resulted in a slight increase in the number of malformed fetuses (3.1 percent in the microwave exposed group, 1.7 percent in the 31°C elevated temperature group). The predominate malformation was cleft palate.

Fertilized Japanese quail eggs were exposed during the first 12 days of embryonic development to an incident power density of 5 mW/cm^2 (SAR = 4.03 W/kg). At hatching on day 18, control and exposed chicks were banded for identification and reared in a conventional manner. During the egg laying period both control and exposed females were mated to exposed and control males for 15 day periods of time and then rotated between groups during the 16 week laying period. Eggs were collected, and fertility and hatchability were evaluated. Fertility was significantly reduced using matings of exposed males with both exposed and control females while hatchability of fertile eggs was unchanged. After 22 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 significantly reduced ($P \leq 0.01$) in the exposed males. Spermatozoal viability and several morphological characteristics in the exposed birds were not consistently different from controls. Relative testes weights were not altered significantly in the exposed males. Histological evaluation of the testes indicated no gross morphological or cellular abnormalities in either control or exposed quail. Studies were conducted to assess reticuloendothelial (RE) and humoral immune function in mature Japanese quail which had been exposed to microwaves of the same duration and intensity described above. Following hatching, exposed quail were reared to 22 weeks of age. Circulating numbers of leucocytes, RE function and primary humoral immunity were evaluated. Leucocytes were elevated significantly in exposed quail (10.17×10^3 versus 8.29×10^3 cells/mm^3). RE function, as indicated by clearance time of intravenously administered colloidal carbon, was not affected ($t_{½}$ in controls was 2.7 min versus 2.8 min in exposed quail). Total hemagglutinins and relative levels of IgG and IgM were not affected by by microwave irradiation. Apparently, RE function and humoral immunity developed normally following irradiation during the embryonic period. However, the reduction in circulating leucocytes suggests a hematopoietic anomaly and potential immune dysfunction.

Research to identify the biological structures that transduce microwave radiation into auditory activity and to investigate possible effects of microwave radiation on the metabolic structure outside the auditory system was performed. Patterns of evoked activity in auditory nerve fibers of rats exposed to pulses of microwave radiation and to acoustic clicks have confirmed the hypothesis of a transduction of microwave energy into mechanical disturbances peripheral to or at the basilar membrane. In addition some evidence for a direct effect of microwaves at the sensory hair cells of the cochleas has been obtained. Patterns of [^{14}C]2-deoxy-glucose utilization at the levels of the inferior colliculus and medial geniculate body of rats with one middle ear oblated have shown that exposure to pulsed microwave radiation can elicit a metabolic response in the auditory system by some mechanism other than conduction of sound through the middle ear.

NOISE BIOEFFECTS

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

Areas under current investigation include: investigation of the correlation between the cochlear potentials and ion movements within the cochlea and their alteration by exposure to noise; examination of the quantitative differences between damage caused by impact and steady state noise when equated for total energy content; identification of the physiological mechanisms corresponding to complex signal analysis (speech) breakdown after slight noise trauma not predictable from simple signal pure tone tests; verification of technical factors affecting the precision of current speech discrimination tests and the development of electronic hardware to correct some of the sources affecting the precision; and noise effects upon the embryo and fetus.

Considerable progress has been made toward understanding the role of ionic permeability of the endolymph-perilymph barrier in normal and noise exposed guinea pigs. In these animals the primary site of noise damage is at the level of the sensory hair cells of the organ of Corti. The suppression of hair cell responses during exposure to intermediate levels of noise was found to be correlated with an apparent reduction in the permeability of the endolymph/perilymph barrier to potassium ions. Similar changes in cochlear potentials were found when the cochlea was treated with tetraethylammonium which is known to suppress the K^+ conductance in excitable membranes. Prolonged exposure to high noise levels results in deterioration of this barrier and a concomitant fall in both the endochlear potential and endolymph potassium concentrations. The electrochemical profile for potassium ions across the hair cell-membrane was measured in normal and noise exposed guinea pigs. The potassium ions are in an electrochemical equilibrium distribution across the basolateral hair cell-membrane and the potassium ions in the endolymph have a high free energy level with respect to the intracellular potassium ions in the hair cells. The electrochemical profile for potassium ions across the hair cell-membrane does not show marked changes in survived hair cells in guinea pigs

exposed to noise. The chloride ion movement across the endolymph-perilymph barrier in normal and pathological conditions is now being studied.

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

Experiments to determine the effect of in utero noise exposure on the conceptus and fetus have continued. The exposure of pregnant CF-1 mice to either semi-continous high level noise (126 dBA jet engine noise) or unanticipated high intensity startling sounds, resulted in embryo-lethality and decreased pregnancy maintenance. This effect did not appear to be related to elevation of plasma corticosterone levels. No teratogenic effects were noted in CF-1 mice in response to noise exposure. Exposure of the CF-1 mouse to noises, whose spectra were coincident with the most acute frequency band of the mouse (18-20 KHz), resulted in significant late stage fetolethality, an effect previously shown by others to be correlated with exogenous catecholamine application. Replication of the experiment in CD-1 mice with concomitant measurement of catecholamine levels in plasma and uteri failed to generate late stage fetolethality. Teratogenic effects, lowered pregnancy rate, excess early stage resorption, and lowered maternal and fetal weight were noted. Plasma norepinepherine/ epinepherine ratio was reversed by noise exposure in late stage pregnancy and uterine norepinepherine levels were significantly elevated during late stage pregnancy in noise exposed CD-1 mice. In another experiment exposure of pregnant guinea pigs to textile mill noise (elevated to 115 dB SPL) caused a significant deterioration in the hearing of the offspring as measured by the brainstem evoked response techniques.

In conjunction with the Laboratory of Behavioral and Neurological Toxicity, effects of maternal noise exposure are being extended into neurobehavioral measures obtained from the offspring (of rats) during adult life.

An improved fiber optic lever using commerically available, economical, and easily assembled components with a 10 A resolation across 10 KHz bandwidth has been developed and will be used to measure ossicular chain motion relative to sound pressure characteristics. A version of the lever optimized for impact measurments of mechanical surfaces was also developed and described in the literature. This version, which should be very useful in diagnosing noise emission from complex machinery, was used as the basis for a successful grant application by others for use as the vibration sensing component of an acoustic intensity meter.

The effect of protracted noise exposure on the cardiovascular function of primates has been examined via an interagency contract. In these experiments Rhesus monkeys have been exposed for periods of up to the one year to noise conditions resembling those found in the community and workplace. The blood pressures of the two experimental monkeys after six months of chair restraint in the quiet were at the

50th percentile with respect to the indirect reference base. However the diastolic pressures had risen to the 99th precentile after six months of noise exposure. Volume changes rather than an increase in peripheral resistance were noted. In the second phase of this study, stroke volume was measured directly and biochemical (blood chemistry, plasma catecholamines and cortisol) parameters were monitored but the results have not been analyzed. In a replication of this experiment using a different primate species (cymologus), essentially the same results were obtained.

As part of an investigation into factors affecting precision of current speech-perception-in-noise tests, an instrument was developed which measures all relevant speech electro-acoustic amplitude parameters. Since provision for generating and modulating white, pink, and speech spectrum noise was also included, the instrument can be utilized to investigate interaction of the masking noise spectra characteristics and intelligibility; including the potential for confounding test scores. Since the major technical capabilities have been assembled in a single instrument for the first time, this development is expected to stimulate further investigation (by others), perhaps leading to progress in assessing the social and economic cost of hearing loss. Progress in quantifying hearing loss relative to the difficulties experienced in everyday noisy environments is presently impeded by the imprecision of conventional methodology and technology. The instrument has been used in this laboratory in assessing intelligibility of speech passed through hearing aids and novel speech transduction devices having less distortion. In these tests, scores obtained with speech mechanically coupled onto the ossicular chain and electronically recovered from the cochlea by differential electrode techniques significantly exceeded those obtained with similar speech passed through hearing aids. Additional tests showed that, where distortion of the peak clipping type was involved, the conventional speech peak equalization methodology acted to confound test scores while use of the long term RMS amplitude measure did not.

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

LIGHT

This program is concerned both with the biological effects of artificial lighting and with the interactions that occur between light and chemical agents in the skin (photosensitization). The beneficial effect of sunlight in the photoactivation of vitamin D precursors in the skin is well known. Cyclical changes in lighting also affect the maturation of gonads in both mammals and man. In addition to these effects sunlight elicits a number of undesirable side effects ranging from erythema ("sunburn") to skin cancer. More recently it has been suggested that artifical light sources, particularly those which have energy spectra that are markedly different from sunlight, may have undesirable side effects. The spectrum of fluorescent lighting, a light source found in both the home and work environment, is grossly distorted when compared to sunlight. For this reason, we have carried out a series of studies to determine whether fluorescent lights have any hitherto unknown biological effects. C_3H mice were selected as the test species because they spontaneously develop mammary tumors and because previous studies have suggest that exposure to certain fluorescent lights decrease their lifespan. Results show that, whereas female C_3H mice housed under daylight-simulating conditions developed mammary tumors in 51 weeks, those exposed to cool-white and pink fluorescent

lights developed tumors in 47 weeks and 42 weeks respectively. Furthermore, the first litters of mice housed under the cool-white and pink fluorescent lights were significantly delayed. The litter sizes of dams exposed to the cool-white fluorescent lights were significantly smaller than those of dams kept under daylight-simulating conditions. In other experiments Sprague Dawley rats born and reared under high pressure sodium vapor lamps had heavier adrenals than those housed under daylight-simulating conditions.

Another adverse effect of light results from its interaction with chemical agents in the skin. The chemical agent may be endogenous (e.g., protoporphyrin), a drug (e.g., sulfonamides, declomycin), topical agent (e.g., p-aminobenzoic acid in sunscreens) or an environmental agent (e.g., polycyclic aromatic hydrocarbons). The combined effect of light and these agents causes skin photosensitization which may take the form of either phototoxicity or photoallergy. While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolite(s), the precise mechanism is unknown. The effect of light on a series of aromatic compounds including two known photosensitizing agents, sulfanilamide and 4-aminobenzoic acid. Results indicate that these compounds give rise to free radicals on irradiation with light at wavelengths above 300 nm. While this finding suggests that free radicals may be responsible for the skin phototsensitizing properties of these agents evidence is now being sought for other possible mechanisms including singlet oxygen formation and energy transfer to biologically important macromolecules.

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

MOLECULAR BIOPHYSICS

The Molecular Biophysics Program is conducting research to understand at the molecular level the interaction of environmental agents with target biological systems including nucleic acids, proteins membranes and microsomal systems. For these studies a number of highly sophisticated spectroscopic techniques, e.g., electron spin resonance and nuclear magnetic spectroscopy, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry, are being employed.

Nucleic Acids:

It is now widely recognized that many mutagenic agents exert their biological effects by modifying DNA. Base pair specific probes are being used that monitor the interaction of mutagens with nucleic acids. The acridines, which bind strongly to DNA, are known to be mutagenic. Spin labeled analogs of 9-aminoacridine have been synthesized and their binding to nucleic acids studied using a number of techniques. Two of these labels were found to have specificity for A-T and G-C base pairs respectively. With the aid of these labels, it was possible to show that histone H_1 binds to A-T base pair rich regions of DNA in the minor groove.

Three spin labeled analogs of actinomycin D have also been prepared. While they exhibited weaker binding to DNA than the parent compound they retain base specific binding and intercalate into G-C base pairs of DNA. In addition, these analogs showed better antitumor properties than the parent drug. We have investigated the stimulation of superoxide formation by these analogs as a possible mechanism of action. These compounds are more effective in stimulating O_2 uptake and the formation of superoxide than the parent drug. In addition, binding studies with human erythrocyte ghost membranes show that these compound interact with membranes and are located in environment which are not accessible to ferricyanide ions. Thus, it seems that the antitumor activities of these compounds may be related to either the increased superoxide formation and/or binding to membranes.

The covalent binding of chemically reduced adriamycin and daunorubicin to DNA has also been examined. Results show that, under identical conditions, one adriamycin molecule is bound per 15 nucleotides whereas only one daunorubicin is bound per 140 nucleotides. These findings may explain why adriamycin induces more DNA damage than daunorubicin as evidenced by an increase in sister chromatid exchange. Enzymatically activated drugs also bind covalently to DNA with identical binding ratios. Results with synthetic polynucleotides show that the binding takes place predominately at guanine bases of DNA. In vivo binding studies show that adriamycin binds to DNA, RNA and proteins, however, binding decreases rapidly with time suggesting an enzymatic repair process is operative. The active alkylating agents derived from these drugs are not known at this time. Other investigations have also shown that several antitumor drugs induce membrane protein conformational changes in erythrocyte ghosts and mastocytoma cells. This finding suggests that the cytotoxic and mutagenic properties of these agents may involve membrane effects as well as interaction with nucleic acids. Recently, Moore has proposed a mechanism for the bioactivation of adriamycin to a covalent binding species. In this scheme, a quinone methide with a carbocation character at C_7 is presumed to be alkylating species. We had earlier suggested that, in addition to the quinone methide, a C_7 free radical intermediate may also act as an alkylating agent. We have recently examined the formation and binding of these species from adriamycin and daunorubicin to DNA. Our studies indicate that the microsomal - NADPH activation produces both one electron (C_7-free radical) and two electron reduction (C_7-quinone methide) products from adriamycin and daunorubicin. Furthermore, 105,000 g supernatant produces only the two electron reduction product. We have further shown that both one electron reduction product and two electron reduction product alkylate DNA.

Superoxide ($O_2^{\cdot-}$) or its toxic metabolites formed from the microsomal-NADPH incubations of anthracyclines have been recently proposed to act as primary toxic agents. Furthermore, these species have also been implicated in the cardiotoxicity of anthracyclines. We have examined the ability of six anthracyclines and DHAQ, (a related anthraquinone compound with demonstrated antitumor activity and cardiotoxicity) to stimulate $O_2^{\cdot-}$ production in rat hepatic microsomes and beef heart submitochondrial particles. Our studies indicate that antitumor activity of anthracyclines is not related to $O_2^{\cdot-}$ production. In addition, intercalated drugs are not effective in $O_2^{\cdot-}$ production and hence cannot as carriers for site specific production of $O_2^{\cdot-}$. Our studies also show that these agents induce peroxidation of rat hepatic microsomal lipid and that highly cardiotoxic agent induce significantly more lipid peroxidation than those agents that are less cardiotoxic. Thus it appears that lipid peroxidation plays an important role in the pathogenesis of cardiotoxicity. Further, α-tocopherol, a known free radical scavanger, offered only a marginal protection against lipid peroxidation induced by these agents.

Reduced glutathione, on the other hand afforded a complete protection against lipid peroxidation.

Membranes:

A large number of chemical agents are known to cause mast cell degranulation with the concomitant release of histamine, serotonin and other pharmacologically active compounds. While the initial event must involve the binding of these agents to the mast cell membrane, little is known about the molecular events that ultimately lead to degranulation. The interaction of compound 48/80 (a classical mast cell degranulating agent) with a number of membrane systems (erythrocyte ghosts, mastocytoma cells, purified mast cells) has been studied in an attempt to elucidate the mechanism of histamine release. The fluidity of the mastocytoma cell membranes was found to be significantly greater than that of normal mast cells. Compound 48/80 did not affect the fluidity of the membrane but did increase the number of binding sites available for spin-labeled stearic acids. While other experiments suggested that 48/80 induced a conformational change in the membrane proteins of erythrocyte ghosts, the significance of this finding and its relevance to the effects of this agent on mast cells is unclear. With the aid of fluorescence microscopy it has been possible to show that, at low concentrations, 48/80 binds to sites in the cell membrane of most cells and mastocytoma, while at high concentrations binding also occurred to intracellular anionic sites (DNA in the mastocytoma cells and granules in mast cells).

The effect of 2450 MHz microwave radiation at 8.2 mW/cm^2 and 41 mW/cm^2 SAR (specific absorption rate) on mast cells has been studied. Histamine secretion was stimulated via three different biochemical pathways using compound 48/80, concanavalin A and ionophore A23187. The results showed that microwave radiation did not affect mast cells or histamine secretion induced by any of these stimulators. It has been suggested that microwave may induce local temperature gradients across cell membranes. By measuring heat-induced inhibition of histamine secretion, we have shown that, under our exposure conditions, the cells were heated between 0.4-0.9°C above ambient temperature when exposed for 10 min at 41 mW SAR.

Mast cells have been used as a tool to study protoporphrin-induced phototoxicity in a eukaryotic cell. It has been found that 48/80 induced histamine secretion is inhibited in the presence of protoporphrin and low intensity light. The inhibition may be due to crosslinking of membrane proteins caused by photoxidation of susceptible amino acid residues. This inhibition is irreversible and, once initiated, continues in the dark although it does not develop into lysis. In contrast, protoporphrin can induce lysis in mast cells under the influence of high intensity light. The molecular basis for this effect and presumably the phototoxic inhibition of secretion may be similar to that described for erythrocytes under the influence of erythropoietic protoporphoria.

Circular dichroism studies of erthrocyte ghost membrane proteins have shown a significant decrease in the optical rotation at 224 nm after protoporphrin and high intensity light exposure. This indicates that protoporphrin phototoxicity causes a decrease in the α-helical structure of the membrane proteins. This technique has made it possible to study more precisely the development of protoorphrin phototoxicity in erythrocyte membranes and the molecular pathways involved.

Anthracene phototoxicity is also being investigated. Erytnrocytes exposed to anthracene show up to 78% hemolysis after 90 min illumination. The effect is

concentration and time dependent and does not occur in the dark. Recent experiments, using anthracene-derivatized fatty acids, have shown that anthracene is much more toxic to erythrocytes when covalently bound close to the polar end of the fatty acid molecule (2-anthroyl-palmitic acid). Anthracene attached to the 16th carbon of palmitic acid was not effective as a photosensitizer. These data indicate that the site of photosensitization by anthracene is near the interface region of the erythrocyte membrane. Future plans include circular dichroism studies of anthracene and anthracene-derivatized fatty acids and the effect of D_2O. The phototoxic properties of anthracene and anthracene-derivatized fatty acids will be investigated using the rat peritoneal mast cell system.

The effect of 2450 MHz microwave radiation on the membrane proteins of human erythrocyte ghosts has been investigated using ultraviolet circular dichroism spectroscopy. A specially constructed waveguide inserted into a spectropolarimeter allowed continuous recording of the optical activity due to secondary structure in membrane proteins. Microwave-induced conformational changes in protein α-helical structure have been compared with the effects of conventional heating.

The ultraviolet circular dichroism data indicate that high levels of 2450 MHz microwave radiation (600 mW/g, specific absorption rate) induce decreases in α-helical conformation that may be due both to thermal vibrations and to increased strain on the intramolecular hydrogen bonds that maintain secondary structure. The latter effect may result from differential intramolecular interactions with the oscillating electric field. Spectrin (bands 1 and 2) isolated from the ghosts was more sensitive to microwave irradiation than intact ghosts, and spectrin-depleted vesicles were the least sensitive. The data, therefore, indicate that the α-helical conformation of spectrin is altered by high levels of microwave radiation.

The effect of 2450 MHz microwave radiation on erythrocyte membrane protein conformation and calcium binding was studied with the fluorescent probe, 1-anilino-8-naphthalenesulfonate (ANS). Using fiber optic cables, excitation light was delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a fluid-filled, temperature-controlled waveguide. Fluorescence was collected using an identical cable and transferred through appropriate filters to standard detecting, amplification and recording devices.

Microwave radiation at specific sbsorption rates of 10 and 200 mW/g had no effect on the binding of the fluorescent probe, ANS, to the erythrocyte membranes. Concentration response curves of increased fluorescent intensity versus calcium concentration also showed no microwave influence on calcium binding between $2.0 - 10.0 \times 10^{-4}$M. In addition, experiments studying fluorescence energy transfer between intrinsic tryptophan residues and membrane bound ANS showed that intermolecular distances between donor and acceptor were also unaffected by microwave radiation.

Proteins:

Binding of metal ions Cu^{2+} and Ni^{2+} to albumins from different animal sources were studied using several physical techniques. The results from chloride ion NMR probe experiments are in good qualitative agreement with our previous studies using a cupric ion selective electrode. The chloride ion NMR studies seem to suggest a strong similarity between the Cu^{2+} and Ni^{2+} binding to different albumins and the relatively high motional freedom at the N-terminal copper site.

The circular dichroic spectra observed below 600 nm for Cu^{2+} binding to human serum albumin (HSA) were absent in the case of Cu^{2+} binding to bovine serum albumin. The binding site in HSA composed is of 4 nitrogen ligand in the plane of the Cu^{2+} complex.

More than 12 histidine C-2 and C-4 protons were observed in the 400 MHz proton magnetic resonance spectrum of HSA. Attempts will be made to make individual assignments to the specific His residues. It has been possible to label HSA with 2,6-dinitro-4-trifluoromethyl benzene sulfonyl group and observe the ^{19}F NMR spectrum of the bound label. Further studies are being made with the labeled protein. The label is presumed to be at the acetylation site of HSA by aspirin. Fluorescence spectroscopic studies have been made with modified HSA. The results obtained so far show that the fluorescent label at the lone sulfhydryl group, the bilrubin binding site and the lone tryptophan are so oriented that from the energy transfer it is possible to make some critical distance measurements. Circular dichroism studies showed induced dichroism for the bound label indicating a chiral environment. A tetrapeptide with Asp-ALa-His-Lys-OMe has been synthesized. Efforts are being made to characterize and use it as a model for N-terminal Cu^{2+} site of albumin.

Microsomal Systems:

It is now generally accepted that the toxicity of many xenobiotics results from the generation of highly reactive chemical species including free radicals. The aims of this project are to: discover new free radical pathways of metabolism; investigate the subsequent reactions of these free radicals with macromolecules and oxygen in order to understand their biochemical fate; develop approaches that permit the clear delineation of the role of free radicals in xenobiotic toxicity. Three new classes of free radical metabolites have been discovered: semiquinone-imine radicals (actinomycin D, dichloroindophenol and serotonin), triphenylmethyl carbon-centered radicals (gentian violet) and sulfur dioxide-derived radicals (SO_2^- and SO_3^-). In addition, a number of free radical reactions with direct biochemical consequences have been examined. These include: the oxygen inhibition of nitro and azo reductases and the associated superoxide formation; the oxidation of NADH to a free radical by the serotonin semiquinone-imine; the formation of a polymeric material from anthracycline free radicals which has an electron spin resonance spectrum like melanin. Finally, a new type of metabolite, identified as an arachidonic acid adduct of nitroso compounds, has been discovered during an investigation of the mechanism of prostaglandin synthetase. Prostaglandin synthetase has a peroxidase activity which appears to catalyze free reactions identical to those catalyzed by horseradish peroxidase. The free radical intermediates of benzidines, hydrazines, aminopyrine and SO_2 are being studied in these systems.

Personnel

The laboratory is organized into three separate Work Groups: Non-ionizing Radiation (Dr. Donald I. McRee, Head), Noise Bioeffects (Dr. Colin F. Chignell, Acting Head) and Molecular Biophysics (Dr. Colin F. Chignell, Head). Dr. Hirohiko Mori (Visiting Associate) joined the Noise Bioeffects Workgroup in May 1980. The Molecular Biophysics Workgroup had two new additions Dr. Carolyn Mottley (IPA) in August, 1980 and Dr. Ann Motten (NRS Postdoctoral Fellow) in June 1980.

Dr. Colin F. Chignell: Adjunct Professor of Pharmacology, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, NIEHS representative, US-USSR Cooperative Program on Photobiology; member of American National Standards Institute Z-311 Committee on the Biological Effects of Nonionizing Radiation. Managing Editor, Journal of Biochemical and Biophysical Methods; Editorial Board member: Molecular Pharmacology, Proceedings of the Society for Experimental Biology and Medicine, Environmental Health Perspectives, Chemico-Biological Interactions. Invited speaker at the symposium on Structure Activity Correlation as a Predictive Tool in Toxicology, Raleigh, N.C., February 10-12, 1981. Seminars at the Department of Chemistry, Duke University, Durham, N.C. on October 17, 1980 and the Department of Pharmacology, University of North Carolina, Chapel Hill, N.C. on March 20, 1981.

Dr. Michael J. Galvin: Adjunct assistant professor, NCSU, Graduate Committee, Cindy Hall, NCSU. Member Shock Society. Participant, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation.

Dr. Ronald P. Mason: Invited speaker American Association for the Advancement of Science, Toronto (1981); invited speaker Biophysics Department Roswell Park Memorial Institute; invited speaker Radiation Laboratory, University of Notre Dame; invited speaker National Biomedical ESR Center, the Medical College of Wisconsin; invited speaker Laboratory of Toxicology, Harvard School of Public Health; invited speaker Imperial Chemical Limited, Manchester, England; invited speaker to meeting on "Free Radicals, Lipid Peroxidation and Cancer", London, England.

Dr. Donald I. McRee: Adjunct Professor, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; Organized workshop and headed a delegation of scientist to the Soviet Union; Edited published proceedings of 3rd US-USSR Workshop on Physical Factor in the Environment; NIEHS representative on Inter-departmental Radiation Advisory Committee (IRAC) on Biological Effects of Non-ionizing Radiation; representative for DHHS on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission lines (organized by DOE); representative of American National Standards Institute C-95 Committee on Safety Standards of Non-ionizing Radiation; appointed to National Research Council Committee on Biological Effects of Non-ionizing Radiation (reviewed report by PAVE PAWS panel); member of the Graduate Advisory Committee, Cindy Hall, NCSU. Invited participant on National Research Council Workshop on "Mechanisms Underlying Effects of Long-term, Low-level 2450 MHz Radiation on People", moderator of session on immunlogical effects;. Appointed a member on a Scientific Panel by the Bonneville Power Administration to develop an epidemiological study of high voltage transmission line workers. Elected Board member of the Bioelectromagnetics Society; Appointed member to IEEE's Committee on Man and Radiation (COMAR); Reviewers of Contract proposals for EPA, NSF, and Army Research Office; Reviewer of technical papers for EPA, Bioelectromagnetic Society, Journal Environmental Health Perspectives, and Radiation Research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50005-07 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

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

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The long range purpose of this project is to increase our understanding of the basic mechanisms of electrolyte movement across the endolymph-perilymph barrier in the guinea pig cochlea under normal conditions and under the influence of physical and chemical agents. The aim of current work is (1) to reveal electrolyte movement (Na^+, K^+ and Cl^-) across the endolymph-perilymph barrier after exposure to intermediate level of noise and (2) to correlate alteration of the ion permeability of the cochlear partition with changes in sensitivity of the auditory organ.

PROJECT DESCRIPTION

METHODS EMPLOYED: Healthy guinea pigs were anesthetized with sodium pentobarbital. The Cl^- activity of the endolymph and the endocochlear potential (EP) were measured with a double-barreled Cl^- selective liquid membrane electrode. The Cl^- activity of the perilymph and the sound-evoked cochlear responses were measured with a pair of Ag/AgCl electrodes positioned in the scalae vestibuli and tympani of the basal turn of the cochlea. Perfusion of the perilymphatic space was carried out at the rate of 2 µl/min for period of 60 min. The perfusates used were (1) normal mammalian Ringer's solution (Na,155mM; K,5mM; Cl,146mM), (2) K^+-deficient Ringer's solution (Na, 150 mM; K, 5mM; Cl, 143 mM) (3) acetate-Ringer's solution (Na, 150 mM; K, 5 mM; Cl, 9 mM) and (4) choline-Ringer's solution (Na, 13 mM; K 5 mM; Cl 148 mM). At the end of perfusion K^+ and Na^+ concentrations in the endolymph and fluids in the perilymphatic space were collected and determined with a helium-glow photometer. Their Cl^- concentrations were measured using electrometric titration techniques.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potentials. The EP showed a slight increase (105%) during perfusion with normal Ringer's solution. The mean loss of the EP after perfusion with K^+ deficient Ringer was 40%. Perfusion with acetate-Ringer's or choline Ringer's solution resulted in a slight suppression of the EP. The CM was slightly suppresssed as perfusion continued but the loss of CM was less than 10%. Perfusion with K^+-deficient Ringer's solution decreased CM to 40%. The mean suppression of the CM was 33% and 54% in perfusion with acetate-Ringer's solution and choline Ringer's respectively. The AP was gradually suppressed during perfusion with acetate-Ringer's, choline-Ringer's or K^+-deficient Ringer's solution.

2. Electrolyte Concentrations. The mean ($\pm$ s.d) Cl^- concentration in the endolymph was 131.0 $\pm$ 5.6 mM in non-perfused cochlea. The mean ($\pm$ s.d) concentrations of Cl^- in the perilymph of the scala vestibuli and the scala tympani were 127.8 $\pm$ 5.3 mM and 128.6 $\pm$ 5.1 mM respectively. Perfusion with normal Ringer's solution did not alter Cl^- concentration in endolymph. However when the perilymphatic space was perfused with acetate-Ringer's, the mean Cl^- concentration in the endolymph showed a considerable decrease (126.7 mM). When the perilymph was replace with choline Ringer's or K^+ deficient Ringer's solution, the Cl^- concentration in the endolymph remained little changed.

On the other hand there was a decrease of K^+ concentrations (147.7 mM) and a substantial increase of Na^+ concentrations (2.2 mM) after perfusion was carried out with K^+ deficient Ringer's solution.

We plan (1) to carry out measurement of Cl^- concentrations of the cochlear fluids in noise exposed guinea pigs under similar conditions to those used in control experiments and (2) to study mechanisms of Cl ion movement across the endolymph-perilymph barrier in normal and pathological ears.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to various electrolytes has been frequently hypothesized but he supporting data are rather limited. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ears under the influence of physical and chemical agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50014-11 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Microwave Exposure Systems and Microwave Dosimetry

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

PI: Donald I. McRee Research Physicist LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

It is the objective of this project to develop and obtain microwave exposure systems for bioeffects research and to develop and test techniques for measuring energy absorption. Waveguide systems for exposing cell preparations, which allow stirring during exposure have been developed. These systems also have the capability of controlling temperature at any desired level between 10°C and 60°C. The systems operate at 2450 MHz and are capable of providing specific absorption rates (SAR's) from 0 to 100 mW/g using continuous wave radiation and 0-30 mW/g using pulse wave radiation. These systems have been modified further so that analytical on-line monitoring of biological parameters can be performed. Techniques which can now be used in detecting biological changes include circular dichroism and fluorescence. A circularly polarized waveguide system for long-term studies has been obtained and is being calibrated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to develop microwave exposure systems for biological material which have well-defined, field characteristics; and to develop dosimetric techniques for energy absorption. At present, it is difficult to measure accurately low level microwave fields and to determine the mechanisms by which these fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistors, and liquid crystals are some of the techniques which will be investigated. Other techniques such as implantable electric field probes will be evaluated as they become available.

MAJOR FINDINGS AND PROPOSED COURSE: Waveguide exposure systems have been developed for cell systems which allow stirring of the medium while being exposed to 2.45-GHz microwave radiation. These systems provide specific absorption rates (SAR's) of 0-100 mW/g for continuous wave irradiation and 0-30 mW/g for pulse wave irradiation.

A waveguide exposure system has been modified so that instrumentation for monitoring fluorescence in erthrocyte ghost membranes can be utilized before, during, and after microwave radiation. Using non-fluorescent, UV-transmitting fiber optic cables, excitation light of specific wavelengths can be delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a the fluid-filled, temperature-controlled waveguide. Fluorescence can be collected using an identical cable and transferred through appropriate filters to standard detecting, amplification and recording devices. A waveguide exposure system was also designed so that it can be inserted into a spectropolarimeter for monitoring the optical activity of proteins of human erythrocyte ghosts using circular dichroism - spectroscopy. These original designs of microwave exposure systems enables the use of powerful analytical techniques using fluorescence and circular dichroism.

Although the tasks defined in the project will continue during the next fiscal year, the project as a separate task will be terminated and included in the methods employed sections of other individual projects.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of exposure systems with well-defined field characteristics and/or capability of accurate specific absorption measurements will enable the Institute to perform accurate quantitative studies on the effects of microwave radiation on biological systems at frequencies ranging from 1-10 GHz. Techniques to determine the amount of energy absorbed will provide the capability of evaluating the data in terms of thermal or specific microwave effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-07 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Microwaves on Neural Response

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

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Howard Wachtel	Consultant	LEB	Duke University

COOPERATING UNITS (if any)
Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Nonionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 adsorption rates (SAR's) of 10 mW/g and above. No differences in rundown time were observed between the continuous wave and pulse wave exposure using the same average SAR. No difference in loss of vitality was observed for synchronous microwave pulses with the peak of the compound action potential, quiescent period, or asynchronous pulses which occurred at random periods in relationship to the compound action potentials. Exposures of frog sciatic nerves to sine-wave modulated 2.45 GHz microwaves at frequencies of 16 and 32 Hz are in progress. Preliminary results indicate that a higher average SAR is required to produce a loss of vitality in the exposed nerve then was necessary for CW and pulse microwaves.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the sciatic nerve of the frog, lobster ganglia, abdominal ganglion of the Aplysia, and saphenous nerves of cats will be exposed to CW, pulsed and modulated microwave radiation in the specific absorption rate (SAR) range of 0-100 mW/g. The effects of the radiation on nerve function and the mechanisms of interactions involved in any changes will be investigated.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of isolated from sciatic nerves to pulsed 2.45-GHz microwave radiation at a SAR of 10 mW/g has been carried out in the same waveguide exposure system as previously reported for continuous wave experiments. In order to determine if pulse wave radiation directly stimulated a pulse width of 10 μsec were used. No changes in stimulation threshold were observed in the exposed nerves when compared to control nerves. Nerves were also exposed to microwave pulses synchronized with the compound action potential (CAP) in one set of experiments and in the "quiet" period (between CAP's). The microwave pulses were 1.5 μsec apart with an average SAR of 10 mW/g. No differences in rundown of the nerves over that obtained with continous wave exposure at 10 mW/g were observed. These results indicate that the rundown of the nerves depends on the average specific absorption rate (SAR) and is independent of the pulse position relative to the CAP or whether continuous wave radiation or pulse wave radiation is used. Exposure of frog sciatic nerves to sine-wave modulated 2.45 GHz microwaves at modulation frequencies of 16 and 32 Hz are in progress. Preliminary results indicate that a higher average SAR is required to produce a loss of vitality in the exposed nerve than was necessary for CW and pulse microwaves. This finding is important in that is appears that the effect is dependent upon the length of time the electric field intensity is above a given threshold level.

Additional experiments using sine-wave modulated 2.45 GHz microwaves will be carried out in the future. Experiments using labelled ions to measure ionic transport and drugs which open and close membrane channels will also be performed in order to obtain information on the mechanism of action of the microwaves with the nerves.

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 the 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.: The effects of microwave radiation in the vitality of isolated from sciatic nerves. Radiation Research 82: 536-546, 1980.

McRee, D.I. and Wachtel, H.: Pulse microwave effects on nerve vitality. Bioelectromagnetics (In Press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50017-08 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of 2450 MHz microwaves on Embryonic Development, Immunology, and Fertility

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

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	J.P. Thaxton	Consultant	LEB	NCSU
	C. R. Parkhurst	Consultant	LEB	NCSU

COOPERATING UNITS (if any)

Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.0	1.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Fertilized Japanese quail eggs were exposed during the first 12 days of embryonic development to 2.45 GHz microwaves of an incident power density of 5 mW/cm^2 (SAR = 4.03 W/kg). The quail were allowed to mature and were mated over a 16-week period. Fertility was significantly reduced using matings of exposed males with both exposed and control females while hatchability of fertile eggs was unchanged. Spermatozoal numbers and motility in semen samples which were collected manually were significantly reduced ($P < 0.01$) in the exposed males. Other eggs exposed as described above were reared to 22 weeks of age. Circulating numbers of leucocytes, reticulo endothelial function, and primary humoral immunity were evaluated. Leucocytes were elevated significantly in exposed quail (10.17×10^3 versus 8.29×10^3 cells/mm^3). No other indices were significantly different. In addition, the hematological and biochemical effects of microwave radiation (10 mW/cm^2) on pups from dams exposed during days 4 thru 20 of pregnancy are being examined. No data is available from this study.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHOD EMPLOYED: A. The first objective of this project is to determine the effects of 2450 MHz CW microwave radiation on embryonic development of Japanese quail and the subsequent growth, reproduction, biochemistry, immunological response of the maturing quail. The fertilized Japanese quail eggs were exposed to an incident power density of 5 mW/cm^2 (specific absorption rate = 4.03 mW/g) for 24 hours per day for the first twelve days of development. The exposures were carried out in an anechoic chamber, which was environmentally controlled so that the eggs were maintained at optimum incubation conditions (37.5°C and 60% humidity).

B. The second objective of this project is to determine the effects of 2450 MHz CW microwave radiation on the development of rat pups. The parameters examined include growth, biochemistry, hematology, and immunological responsiveness of the maturing rats. Pregnant rats were exposed to an incident power density of 10 mW/cm^2 (specific absorption rate = 2.0 mW/g) for 3 hours per day from day 4 thru 20 of pregnancy. The exposures were carried out in an anechoic chamber which was environmentally controlled.

MAJOR FINDINGS AND PROPOSED COURSE: A. This study was conducted to assess reticulo-endothelial (RE) and humoral immune function in mature Japanese quail which had been exposed to microwaves during embryogeny. Quail embryos were exposed continuously to 2.45 GHz at an incident power density of 5 mW/cm^2 (SAR=4.03 mW/g) during the first 12 days of the embryonic period. Following hatching, exposed quail were reared to 22 weeks of age. Circulating numbers of leucocytes, RE function and primary humoral immunity were evaluated. Leucocytes were elevated significantly in exposed quail (10.17×10^3 versus 8.29×10^3 cells/mm^3). RE function, as indicated by clearance time of intravenously administered colloidal carbon, was not affected ($t_{1/2}$ in controls was 2.7 min versus 2.8 min in exposed quail). Total hemagglutinins and relative levels of IgG and IgM were not affected by microwave irradiation. Apparently, RE function and humoral immunity developed normally following irradiation during the embryonic period. However, the reduction in circulating leucocytes suggests a hematopoietic anomaly and potential immune dysfunction.

Following hatching, the exposed and non-exposed quail were reared to 6 weeks of age. Similar parameters are being determined in 6 week old quail. Total circulating erythrocyte and leucocyte numbers, and total hemoglobin (gm% and hematocrit) were determined following multiple bleedings (days 0,3,6,9) in order to evaluate hematological compensation. Total leucocytes and hemoglobin were similar in the exposed and control quail at all sampling times. Erythrocyte numbers were similar in the males at all sampling times; however, on day 9, the exposed females exhibited higher RBC numbers than the control females (3.18 vs 2.55 x 10^6/mm^3, respectively). Although all four groups demonstrated decreased hematocrit due to sampling, no differences were noted between exposed and control quail. These data suggest that microwave radiation of quail during embryogeny influences hematologic capacity of sexually mature quail.

B. Pregnant rats were exposed to microwave radiation during days 4 thru 10 of pregnancy at an incident power density of 10 mW/cm^2. Pups were foster mothered on day 2 post partum and blood samples were obtained from pups at 2,10,15,20 and

30 days postpartum. Circulating numbers of leukocytes, erythrocytes, and differential white celltypes determined. In addition, hematocrit and hemoglobin levels were measured. No data are available from these studies.

FUTURE PLANS: A. Research will continue using the Japanese quail as a test system. Since spleen and bursa of Fabricius in the female adult quail (22 weeks old) were affected by microwave exposure in ovo, immunological studies on 22-week-old quail will be performed. The studies will be expanded to include 6 and 12 week old quail. Longevity studies will also be carried out on the quail exposed in ovo. The average life span of Japanese quail is approximately two years. Normal housing and mating conditions will be maintained throughout the lifetime and reproduction capacity monitored.

B. This project will be completed and extended to studies on the immune capacity of pups from microwave exposed dams.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The question of whether exposure to microwaves during embryonic development has any effect on the development process and function of the organism after maturity must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-06 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
OTHERS:	Blake Wilson	Senior Electrical Engr.		RTI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.65	.70

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Results of behavioral and audiological tests with humans imply that ability to understand speech and other complex signals (particularly in a noisy listening situation) suffers deterioration from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a complex interaction occurs between the various levels of the auditory nervous system and that functionally different receptors of the auditory end organ play a vital role in "sharpening" the sensory process. The objective of this study is to use the rapid signal analysis ability of minicomputers to determine the auditory response to speech and speech-like signals including pseudorandom noise under normal conditions and conditions of auditory fatigue. Auditory fatigue and recovery process of single nerve fibers to speech and speech-like stimuli will be studied. The requirement in high speed data throughput for nearly absolute phase matching of input acoustical and output physiological signals, and for accurately processing neural pulse trains necessitated the design and building of several unique complex peripheral devices. These are presently being debugged. 228

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to produce speech-like inputs most efficiently, pseudorandom noise which has been amplitude modulated by sinsusuoidal signals is presented to the animal. The pseudorandom noise approximates the constantly changing frequencies of speech while the sinusoidal modulation simulates the varying intensities. When the sinusoidally modulated pseudorandom stimulus is presented to the animal the auditory nerve response is simultaneously recorded in the form of a cycle histogram, the cycle being locked to the period of pseudorandom noise. After summing the histogram many times to ensure statistical validity, cross-properties between the output and the known input, which represent the response characteristic of the peripheral auditory system, can be determined.

1. In order to obtain the temporal characteristics of the input noise a high-speed analog-to-digital converter (ADC) system capable of converting at speeds up to 40-50 kHz was necessary. Three channels (at roughly the same high speed) are needed for this and other planned experiments. Since no commercially available ADC system with a PDP-11 interface with sufficiently high speed existed, it was necessary to design and construct one around commercially available high-speed ADC modules and interfacing boards. This project was a cooperative venture involving Computer Engineering, BB, our electronic consultant, and LEB personnel.

2. For acquiring the histogram data, a special high-speed clocked pulse counter interfaced directly with the computer was designed and built on a commercially available interface board by LEB personnel with design guidance from Computer Engineering, BB. As with the ADC, the special design was primarily necessitated by the 40-50 kHz data rate.

3. As an intrinsic adjunct to any high-speed ADC system, a low-pass sharp-cutoff filtering system must be provided in order to prevent aliasing of high frequencies. Phase matching requirements of such filtering systems when time dependent cross properties are to be measured far exceed those normally available filters. State of the art elliptic filters providing a higher maximum data rate and increased phase accuracy became available last year and were built into the system by LEB personnel.

4. Since the binary pseudorandom noise conventionally used to test auditory system function was recently shown to generate 2nd order distortion of the same type as the auditory system, a computer software based system for generating trinary pseudorandom noise was developed by LEB personnel in conjunction with an expert (consultant) on digital filtering.

5. An electronic (hardware) auditory nerve simulator (analog in, digital pulse train out) was developed in order to facilitate shakedown and evaluation of various strategies for computerized acquisition of nerve fiber tuning curves. This system is much more efficient one than use of live animal preparations.

MAJOR FINDINGS AND PROPOSED COURSE: This project has suffered from the necessity to develop sophisticated electronic hardware due in part to the state of the art nature of the electronic interface mechanisms. As soon as these problems have been resolved, pilot projects involving normal animals to acquire baseline data and establish the resolution capability of the system will proceed; followed by

subsequent testing of noise exposed animals.

Hardware development is 100% complete and initial software development is about 90% complete. Because the project involves a major commitment of resources. It is difficult to say exactly when this series of experiments will begin on a full time basis.

A general purpose transfer function analysis program accepting analog inputs while outputting transfer function magnitude and phase, coherence, input and output power spectra, auto and cross correlation and impulse or step response is in place and operating as designed. Extension of the transfer function program to accept nerve fiber inputs is being pursued as time permits and should be completed by early summer. While the laboratory is still working on a sound transducer whose distortion levels are desirably low, pilot experiments will be possible.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistent in spite of the fact that complex stimuli perceived in a noisey environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

PUBLICATIONS

Cook, R.O.: Use of modern signal analysis techniques and special transducers in assessing rapidly changing signals in the auditory system. In: Proceedings of the Conference "Signal Analysis in the 80's". Available from Engineering Foundation 345 East 47th St., NY, NY, 10017.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-05 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Optical Fiber Motion Detector Systems for Auditory System Measures

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

PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS
OTHERS:	Stan Hutcheson	Director of Technical Services in Engineering of the Child Development Institute	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Only a few techniques are capable of the resolution and frequency response necessary for auditory system motion measures; i.e., 10^{-2} - 10^{2} Angstroms across 100 Hz-20 kHz bandwidths. Previous theoretical and experimental efforts have led to determination of intrinsic performance limits and identification of the parameters relevant to optimization for given objectives. These analyses showed optic lever systems to be competitive with interferometric and optical heterodyneing techniques and better than capacitive probe techniques in resolution and an order of magnitude simpler and less expensive than either. Continuing refinement and explorative efforts have been focused in three areas: experimental determination of the optimum probe size/linear range trade off and development of automatic in site calibration circuitry for umbo/ossicular chain measures; development and testing of high speed differentiation circuitry allowing correlation of mechanical impulses with acoustic emissions from imparted plate and other radiating structures creating occupational noise levels of hearing loss significance; and work on specialized probes for measurement of ossicular membrane vibration.

PROJECT DESCRIPTION

METHODS: Physics, electro optics, optics (fibers), electronic engineering computer modeling, noise measurements.

MAJOR FINDINGS AND PROPOSED COURSE: Intrinsic performance limits of this non-contact displacement measuring technology have been quantified, experimentally verified, and published in a form which facilitates design optimization for a variety of applications. Further performance improvements have been focused on more efficient illumination sources and rejection of the 60 Hz harmonic room lighting since other components have been pushed to theoretical limits. It was also determined that by optimizing electronic differentiator parameters and utilizing recently developed very high speed op amps, the displacement signal had sufficient dynamic range to give high dynamic range velocity and acceleration values. Measures of velocity and acceleration obtained from impacted plates obtained by conventional accelerometers were compared with values obtained by this technology in an experiment be signed to correlate shock and sound pressure level amplitude and waveform. A paper which details this additional capability was published, and others have obtained grants based on using this development as the vibration sensing element of an acoustic intensity meter.

Auditory System Optimization: The small size of the termination of the ossicular chain (umbo) in the center of the tympanic membrane coupled with the relatively large "DC" movements imposed on the ossiclar chain by the middle ear muscles pose serious motion measurement problems. Since working distance and linear range are directly proportional to fiber diameter, use of fibers which are too small allows the spontaneous "DC" motions to carry the umbo beyond the linear range of the lever, while use of oversize fibers results in illumination of areas bigger than the umbo, introducing measurement errors. After analytical determination that the optimum fiber diameter was on the order of 125 microns, fibers of 100, 125, and 200 microns diameter were ordered and probes fabricated. Experimental verification of the optimum probe will be undertaken along with shakedown of electronic circuitry built to allow automatic in situ calibration, an important advantage since the ossicular chain is constantly perturbed by middle ear muscle movements.

Fiber Lever Optimized for Basilar Membrane Vibrations: Little effort has been devoted to this effort during the past year due to the time required to complete other projects. Several fiber manufacturers who say they can produce the 25-40μ fibers and accomplish the necessary 90°, 100μ bend ratios have been located. Several such bundles will be purchased in the near future for evaluation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Shock and vibration transducer: Reduction of harmful noise emissions from machinery and other rotating sources can best be avoided at the design stage provided theory on which predictions of the noise emissions resulting from mechanical shock is available. Frequency response of accelerometers used in shock and vibration measurements is usually limited by linearity considerations to one-third of the resonant frequency of the accelerometer. In addition, for highly sensitive measurements, the added mass of accelerometer may alter the characteristics of the surface motion. And finally, to obtain displacement waveform measurements,

the signal obtained through an accelerometer must be integrated twice with great loss of frequency response and dynamic range. The use of noncontacting fiber particularly where knowledge of displacement waveform characteristics is desirable and in high frequency applications. Others are attempting to construct acoustic intensity meters based on this development. Such meters would contribute significantly to identification of noise emission sources within complex systems, including industrial machinery.

Optimized Ossicular Chain and Basilar Membrane Auditory Motion Transducer: A convenient means for making audio frequency displacement measures of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL) has long been needed in the study of auditory distortion and hearing loss mechanisms. Reliable measures of the motion of the basilar membrane would resolve present uncertainties concerning non-linear plateau effects at high intensities and frequency resolution from high and low frequency slopes of the maximas; these uncertainties block confirmation of hearing theories at the intracochlear mechanical level.

In addition to these specific applications, optic levers have been utilized to measure the dynamic force exerted by muscle myofibrils, as the basis for ultra-sensitive pressure transducers for cardiovascular and other clinical purposes. Although the non-contacting characteristic of optic levers uniquely quailfy them for biological (and many mechanical) applications, application of the technique has previously suffered from lack of a theoretical basis from which design optimization could proceed, and from the relatively short working distances and low detection limits associated with the commerically available models. Since the theoretical relationships have been developed for both laser and Lambertian sources and confirmed by measurement, it is now possible to predict accurately the ultimate resolution for given fibers, bundle configurations and illumination sources. Illumination sources made commerically available within the past year by telecommunications industry suppliers have significantly increased device resolution.

PUBLICATIONS

Cook, R.O. and Hamm, C.W.: Fiber optic lever displacement transducer. App. Optics 18(19): 3230-3241, 1980.

Cook, R.O., Hamm, C.W. and Akay, A.: Shock measurement with noncontacting fiber optic levers. J. Sound and Vibration. 76(3): June 9, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50022-05 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Electronic and Electro Acoustic Devices for Hearing Loss Studies

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

PI: Reginald O. Cook Acoustical Engineer LEB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

1) The purpose of this project is to refine a pneumatically powered impact noise generator whose peak level, rise time, and decay can be experimentally manipulated. Mechanical, acoustic and reliability parameters are being measured currently in preparation for (animal) exposure experiments.
2) Tone bursts are widely used in acoustic research, but a means of phase locking the zero crossing and manipulating rise time was needed, but not commercially available. Design of such a device was undertaken.
3) A closed system electroacoustic transducer having the ability to deliver high level, wide band minimum distortion sounds to the eardrum of guinea pigs has long been needed. Design of electronic circuitry, including predistortion, for use with condensor microphone speakers has been initiated.
4) Purpose is to develop a simple instrument with which significant speech discrimination test parameters can be manipulated. These parameters include peak level, long-term equivalent level, defined pink noise and speech spectrum noise for masking purposes and a means for modulating both.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: 1) Mechanical, electronic, and acoustical engineering concepts were utilized in the design phase, which had been preceded by analytical and experimental investigations of the acoustic characteristics of mechanically generated impacts implicated in hearing losses associated with human exposures.

2) Electronic engineering concepts were utilized in the design of the tone burst shaper.

3) Electroacoustic concepts will be employed in the design of these devices.

4) Electronic design utilizing state of the art electronic devices were employed to assure the desired auditory characteristics.

5) Analog and digital electronic engineering concepts were used to design the simulator.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The pneumatic drive mechanism under test appears to have solved the failure rate problem associated with earlier solenoid operated device. Previous tests with animals indicated physiological parameters associated with hearing impairment (CM, AP) deteriorated in different ways when exposed to equal energy impact vs. continuous noise. Chronic impact exposure experiments will begin soon.

2) The tone burst generator was designed and constructed and is currenly in use in auditory experiments described in other reports.

3) Development of the electronic circuitory for this device will probably be undertaken by RTI on a collaborative basis.

4) The 2nd generation model has been constructed and is currently being evaluated.

5) The nerve simulator was constructed under contract by RTI and is currently been evaluated.

SIGNIFICANCE TO BIOLOGICAL RESEARCH AND PROGRAMS OF THE INSTITUTE: 1) The fact that it is not possible to reproduce impact sounds using conventional audio equipment has resulted in few, if any, well controlled laboratory studies of hearing damage from a type of noise which pervades industry and society. There is strong suggestive evidence that high intensity, short rise time peaks, rather than steady state levels, are the major contributor to hearing loss. Because the temporal summing characteristics of the human auditory system operate over a longer time span than the duration of impact sounds, perceived loudness is not proportional to peak level or to peripheral organ damage possibilities. This effort should make refined studies of hearing loss/impact noise parameters possible.

2) Phase locked tone burst capabilities are necessary where CM and AP waveforms are to be recovered through high speed averaging. Experiments involving correlation of physhophysical and physiological tuning/curves are examples.

3) A closed system electroacoustic transducer having the ability to deliver high level, wide band, minimum distortion sounds to the eardrum of guinea pigs has long been needed. Design of electronic circuitry, including, predistortion, for use with condenser microphone speakers has been initiated. Sound sources with which precise control (i.e., low distortion) of acoustic waveforms at the ear, across wide bandwidth and amplitude can be produced. It is believed that current electroacoustic transducer technology can be exploited to achieve advances in noise exposure research.

4) Current practices in testing of speech materials for the hearing impaired have either relied on peak reading instruments, which bias in favor of more distorted speech, or on expensive computers beyond the reach of many audiology labs or clinics. A simple, inexpensive device will make it possible for many more labs to do competent speech discrimination testing. Speech discrimination is used in comparing initially identical speech after passage through different hearing aids, or other fidelity degrading communication systems.

5) An electronic model simulating the auditory nerve makes testing of computer based data extraction algorithms possible while bypassing the myriad of additional problems associate with preparation and maintainence of live animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50026-04 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Closed System Electroacoustic Transducer for Noise Research

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

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
OTHERS:	Teruzo Konishi	Medical Officer	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project has been incorporated into Z01 ES 50022-05 LEB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50028-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effects of Noise and Drugs on the Electrochemistry of the Cochlea

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Determination of electrochemical gradients across the cochlear hair cell membranes is essential for understanding the biophysical basis of cochlear transduction. The aim of this study is to measure the electrochemical driving force for the movement of K^+ in the cochlear hair cells in normal and noise exposed guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: A group of guinea pigs was exposed to broad band noise of at 115 dBA for 7 to 10 days. A second group of guinea pigs was kept in a quiet environment. Exploration of the organ of Corti with double barreled K^+ selective liquid membrane electrodes was carried out in guinea pigs anesthetized with sodium pentobarbital. After rupture of the round window membrane a double barreled K^+ selective electrode was positioned on the surface of the basilar membrane and was advanced toward the organ of Corti by means of a piezoelectric microdriver. A 3M KCl-agar Ag/AgCl electrode was placed on intact neck muscles and used as a reference. During penetration of the organ of Corti the outputs of the chemical and K^+ potential barrel were continously recorded. The cochlear microphonics in response to 500 Hz tone bursts were also recorded from the K^+ potential barrel and photographed on a running film. Identification of the hair cells was based on a sudden increase of the CM associated with an abrupt rise of the membrane potential. When no increase of the CM was observed at moment of pentration of cell membranes, these cells were categorized as supporting cells. Recording of stable responses for periods longer than 5 sec was used as a criteria of successful impalement of cell membranes.

The external tip diameter of double barreled electrodes was less than 1 μm. The mean slope of K^+ selective electrodes was 37 mv/10 fold change of K^+ activity. The Na:K selectivity constant was 40:1. The response time was less than 3 sec.

MAJOR FINDINGS AND PROPOSED COURSE: The data were collected from 11 and 8 successful pentrations of hair cell membranes in normal and noise exposed guinea pigs respectively. The mean and $\pm$ sd electrical and chemical potential differences between the hair cell and the extracellular fluid of the organ of Corti were -82.4 $\pm$ 18.0 mv and 89.3 $\pm$ 21.1 mv respectively. These respective values in noise exposed guinea pigs were -64.3 $\pm$ 22.1 mv and -92.9 $\pm$ 13.5 mv. These data indicate that in both normal and noise exposed hair cells the K^+ ions are in electrochemical equilibrium between the extracellular fluid of the organ of Corti and hair cells. However the endolymph K^+ ions have an extremely high free energy level with respect to the intracellular K^+ ions of the hair cells (198.2 $\pm$ 10.1 mv in normal guinea pigs and 189.6 $\pm$ 20.8 mv in noise exposed guinea pigs). The mean electrochemical potential difference for K^+ across the hair cell membranes did not show marked changes in guinea pigs exposed to broad band noise when compared with that found in normal guinea pigs. Although CM in the scala tympani or scala media was severely suppressed in noise exposed guinea pigs, the ratio of intracellular to extracellular CM was much larger in noise exposed but surviving hair cells. These results seem to suggest that marked suppression of extracellular CM in noise exposed guinea pigs can be attributable to a decrease of number of surviving hair cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The electrochemical profile for K^+ across the hair cell membrane is a significant physiological mechanism underlying the noise-induced hearing loss. The present study will make a significant contribution to understanding the physiology of noise induced ear damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50030-03 LEB

PERIOD COVERED
Ocotber 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Spin Labeled Actinomycin-D Analogs as Base Specific Probes for Nucleic Acids

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

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS
	R. L. Cysyk	Chief, Drug Metabolism Section	LCP	NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, NCI, NIH

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.45	0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this project is to prepare base specific spin label probes to study binding of chemical carcinogens to nucleic acids. For this purpose, we have prepared spin labeled actinomycin-D to probe G-C-regions in nucleic acids. Binding studies with these labels and DNA suggest that these analogs bind to DNA and their DNA-binding modes are similar but not identical to the parent compound. These analogs inhibited DNA-dependent RNA polymerase and were more active than the actinomycin D against P-388 leukemia cells in vivo. In addition, these analogs are more effective in stimulating O_2 uptake and the formation of O_2^- than the parent drug. Sedimentation viscosity measurements suggest that the binding mode is one of intercalation into DNA. DNA-dependent-RNA polymerase studies using various synthetic nucleotide show that these analogs bind only to guanine-cytosine bases in DNA. Electron spin resonance studies suggest that these analogs also bind to cell membranes.

PROJECT DESCRIPTION

METHODS EMPLOYED: We have synthesized N^2[4-(2,2,6,6-tetramethyl-1-piperidinyl-oxyl)] actinomycin D and related 1,2-diaminoethane and 1,3-diaminopropane derivatives (3-5). Biological studies with these compounds and DNA were carried out using circular dichroism, electron spin resonance (ESR) and thermal denaturation.

MAJOR FINDINGS AND PROPOSED COURSE: Our studies have shown that although these analogs bind weakly with DNA, they have high antitumor activities against P-388 leukemia cells in vivo. Their increased antitumor activities may be due to in vivo metabolism of these analogs and binding to some other sites such as cell membranes. We have investigated the stimulation of superoxide formation by these compounds as a possible mechanism of action. Our findings indicate AMD and its derivatives 3 and 5 stimulate O_2 uptake by 154% and 1273%, respectively. Furthermore, in the adrenochrome assay, which is an indicator of superoxide formation, these analogs (AMD_1, 3 and 5) stimulated the rate of epinephribe oxidation to adrenochrome by 90, 260 and 982 percent respectively. Addition of superoxide dismutase (SOD) inhibited the formation of adrenochrome suggesting that O_2^- was responsible for adrenochrome formation. When bound to DNA, Sato et al. have shown that anthracycline antitumor drugs are substrates for microsomal enzymes. Incubation of AMD, and its derivatives with DNA abolished O_2^- formation by AMD and 3, and reduced the stimulation by 5 by 50%.

Sedimentation viscosity measurements studies with these analogs suggest that they intercalate into DNA. ESR studies indicate that they retain base specific binding to G-C bases. This base specificity is further demonstrated by their inhibition of RNA-dependent polymerase only when G-C containing synthetic polymers are used as primers. ESR studies carried out with human red blood erythrocyte membranes indicate that AMD analogs bind to these membranes.

In conclusion, we have shown that AMD and its spin label analog bind to DNA by intercalation. The binding specificity to G-C bases is retained in N^2-substituted analogs. These analogs, as result of this DNA binding, are also inhibitor of DNA-dependent RNA polymerase reaction. In addition, they stimulate O_2 uptake and drastically increase the formation of O_2^-. Furthermore, they interact with cell membranes. At this time, it is not clear which of these interactions (DNA binding, production of $O_2^-/H_2O_2/OH$, or membrane binding) is responsible for the antitumor and carcinogenic properties of AMD.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the binding of many chemicals to nucleic acids and cell membranes is believed to be responsible for their carcinogenicity or mutagenicity, attempts have been made to understand the toxicity of actinomycin, a known carcinogen, by examining nucleic acid and membrane binding. In addition, production of superoxide was also examined. It is evident from our studies that the toxicity of actinomycin may involve all the above-mentioned mechanisms; however, membrane binding and the resulting inhibition of membrane functions e.g. ion transport, or the initiation of lipid peroxidation due to formation of O_2 may play a more important role than DNA binding in the final expression of toxicity of AMD. This project is essentially complete and no future work is planned at this time.

PUBLICATIONS

Sinha, B.K., and Cysyk, R.L. Mechanism of action of N^2-substituted spin labeled actinomycin D: binding to nucleic acids and erythrocyte ghost membranes. Chem-Bio. Interaction, 34, 367-372 (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50031-04 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Mast Cell Degranulation

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

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHER:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The membranes of both purified mast cells and mastocytoma cells have been studied using spin-labeled probes. The membrane fluidity of the neoplastic cells was significantly greater than that of the normal mast cells. The character of the spin probe was also found to be important in determining the accuracy of fluidity studies. Highly charged probes (such as fatty acids) may report a lower apparent fluidity due to the anchoring influence of their charged end group. Compound 48/80, a mast cell degranulating agent, had no effect on any of the lipid spin labels studied; however it was able to reverse the effect of magnetic interactions between closely adjacent spin labels. This suggests that 48/80 may increase the available membrane binding sites for the spin labels. Compound 48/80 also affected the state of MSL-ghost proteins by causing an increase in the population of highly immobilized label. The effect of trypsin was similar between MSL-ghosts and SL-48/80-labeled mast cells. Fluorescence microscopy has shown that mast cells and mastocytoma cells bind R-48/80 on the plasma membrane at low concentration and at high concentration, the binding also occurred at anionic sites in the cell interior.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat mast cells were isolated according to well established procedures and purified by centrifugal elutriation. The mastocytoma cells were an established cell line. The effect of 48/80 on the environment of 5-doxyl-stearic acid (5-DSA) and 5-doxylmethylstearate (5-DMS) was determined using the method in which the half width of the high field hyperfine extrema was measured at half its height. Rotational correlation times and order parameters were also calculated for the spin-labeled fatty acids. Fluorescence microscopy was carried out with a Leitz Dialux 20 microscope equipped with pleopak incident light fluorescence illuminator.

MAJOR FINDINGS AND PROPOSED COURSE: The rat peritoneal mast cell has been widely studied because of its role in the anaphylactic response and because it is a useful model system for examining the secretory process. We have studied the membranes of rat mast cells and peritoneal and pleural cells and also murine mastocytoma cells using the spin label method and fluorescence microscopy. We have also studied the effect of compound 48/80, a highly potent and efficacious stimulator of mast cell secretory action. For our electron spin resonance (ESR) studies, large numbers of purified mast cells were needed. We have therefore employed the technique of centrifugal elutriation to provide rapid purification of large numbers of cells. Human erythrocyte ghost membranes covalently labeled with a maleimide spin label have also been used to study the interaction of 48/80 with the membrane proteins.

The effect of 48/80 concentration on cell binding was determined by fluorescence microscopy. Rhodamine labeled 48/80 (R-48/80) was studied at various concentrations using both mast cells and mastocytoma cells. Using this technique we were able to observe and photograph the binding of 48/80 to various cell types.

Rat peritoneal mast cells, which constitute 6-10% of the peritoneal and pleural cell population, can be successfully and rapidly purified using centrifugal elutriation. It was necessary to include serum albumin for the cells to retain their ability to secrete histamine. Elutriated mast cells released histamine in a manner similar to cells stored in the presence of albumin.

The ESR spectra of 5 DMS in both mast cells and mastocytoma cells suggested that the label was more highly immobilized in the mast cells than in the mastocytoma cells. In contrast, 5 DSA was very highly immobilized in both mast cells and mastocytoma cells and there was no apparent difference between the two cell types. The motion of the nitroxide group attached to stearic acid was very dependent on the position of the nitroxide with regard to the charged end of the molecule. There was a progressive increase in the motion of the nitroxide as it was located further from the ionized carboxyl group and, presumably further into the hydrophobic region of the cell. The signals from the spin labeled methyl stearates show a similar pattern, however, their motion was not as strongly influenced by the position of the nitroxide. In mastocytoma cells, all of the labels except the 5 DSA were less immobilized than in the mast cells. Also, in each case the nitroxide attached to the acid was more highly immobilized than its counterpart attached to the ester. However, this difference decreased as the nitroxide group was moved further from the charged end of the molecule.

The correlation times derived from spin labels in mast cell depleted leukocytes indicate that mast cell membranes are similar in fluidity to the other assorted white blood cells found in the rat peritoneal cavity.

Our data show that the apparent membrane fluidity increases as the reporter group is located further into the hydrocarbon region and further away from the charged end of the molecule. This finding is consistent with the current theories regarding membrane structure in that the most molecular ordering occurs at the surface and interface regions, presumably due to the presence of peripheral proteins and the glycerol backbone of phospholipids. In both mast cells and mastocytoma cells, the molecular ordering as reported by the 5 DSA was similar. However, the rotational correlation time (and presumably the membrane fluidity) increases very rapidly in the mastocytoma cells as reported by the signal from the 12th carbon, and there is little change from the 12th to the 16th carbon. In the mast cell, the increase in fluidity was more gradual and the abrupt change came between the 12th and the 16th carbon atoms. This effect was more pronounced with the acid derivatives, although the ester derivatives also showed a similar pattern. This finding indicates a fundamental difference between the mast cell and the mastocytoma cell membrane; however its significance if any, in explaining the neoplastic character of the mastocytoma cells, remains unclear.

The difference in motion seen between the acid and ester derivatives was greatest when the nitroxide was placed nearest the acid or ester group (5-DMS, 5 DSA), and diminished as the nitroxide was situated further away (16 DMS, 16 DSA). This was seen with both mast cells and mastocytoma cells and is probably due to the stronger "anchoring" effect of the acid moiety. The charge on the stearic acid therefore provides an additional constraint on the motion, and consequently, the apparent membrane fluidity is lower when the reporter group is situated close to its influence. If it can be assumed that the acid and the ester have the same orientation within the membrane, then the stearic acid probes were reporting artificially low fluidities for both the 5-nitroxide and the 12-nitroxide derivatives.

The current study shows that the mastocytoma cells are probably more fluid in all regions of the membrane. The 5 DSA was the only probe to report a similar fluidity for both cell types, however this was probably due to the strong influence of the charged group anchoring the molecule rather than the actual condition of the membrane. The 5 DMS probe suggests that mast cell membranes are indeed more rigid at the interface and this difference persists into the hydrophobic region of the membrane. This study shows that the use of 5-doxyl stearic acid to compare different membrane types can lead to erroneous results and that several different probes should also be included in a complete study of membrane fluidity.

There was no effect of 48/80 on stearic acid and ester spin labels incorporated into either mast cells or mastocytoma cells; and the apparent molecular motion of the spin labels was not changed even at 48/80 concentrations far exceeding that which was maximally active. The effect of spin label concentration in the membrane has been studied. When the cells were incubated with spin label concentations higher than 2.5×10^{-5} M, the extrema of the labeled cells were broadened, presumably due to magnetic interactions between closely adjacent nitroxide

molecules. The data showed that the effect of 48/80 on the spectra was only apparent in cases where there were magnetic interactions present. Compound 48/80 reduced the magnetic interactions and the spectra resumed a normal shape. There was no effect of 48/80 on cells in which the membrane concentration of label was magnetically dilute.

Although compound 48/80 precipitates a major molecular rearrangement in mast cell membranes, it was not possible to demonstrate any effect of this agent on bound stearic acid and ester probes which were magnetically dilute. Therefore, the absence of any effect by 48/80 on the labels suggests strongly that its action does not result in a permanent major fluidity change among the membrane lipids or at the interface.

The effect of 48/80 on high concentrations of the stearate spin-label in the mast cell membrane indicates that 48/80 increased the distance between interacting spin-labeled molecules, thereby reducing intermolecular magnetic interactions. One possible explanation for this observation is that compound 48/80 increases the number of binding sites for the spin labels. We have previously suggested that 48/80 may act by causing a partial denaturation of the membrane proteins. If this mechanism is correct, the denatured membrane proteins may provide the additional binding sites which are generated by 48/80.

Spin-Labeled Ghosts: We have spin-labeled ghost membranes using a maleimide derivative (MSL) which covalently binds to membrane proteins via their sulfhydryl groups. We have found that 48/80 causes an increase in the population of more highly immobilized spin labels. These results are direct evidence of a membrane protein interaction by 48/80. We have also found that treatment of MSL-ghosts with trypsin causes rapid destruction of the highly immobilized sites. Similar treatment of purified mast cells labeled with spin-labeled 48/80 yields the same result. These data indicate that 48/80 binds to a general site on cell membranes which may be similar to that of the MSL and is probably a protein.

Fluorescence Microscopy: Compound 48/80 was covalently labeled with the fluorescent moiety, rhodamine. At low concentrations rhodamine 48/80 (R-48/80) binds to the plasma membrane of both mast cells and mastocytoma cells. In some cases we have observed "patching" of the fluorescence intensity which would indicate aggregation of the receptor sites. At high concentration, R-48/80 bound to anionic intracellular structures. The R-48/80 apparently increases the membrane permeability, allowing access to the cell's interior. In mast cells, R-48/80 binds to the basophilic granules, whereas in mastocytoma cells it binds to the nucleus.

We have made a detailed study of mast cell and mastocytoma cell membranes in order to understand better the underlying mechanisms behind non-cytotoxic histamine secretion. We have found significant differences between their membrane fluidities. We have also shown that mast cell membranes are similar in fluidity to that of the other normal cells found in the peritoneal and pleural cavities and that this fluidity is unaffected by 48/80. This indicates that the ability to secrete histamine is unrelated to membrane fluidity. In addition we have shown by fluorescence microscopy that 48/80 binds to the outer membrane at low concentrations and to inner constituents at higher levels.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunological stimulation of histamine secretion via IgE antibody-antigen complex formation mediates the symptoms of asthma, hay fever and anaphylactic shock. In addition to this reaginic response, histamine secretion can also be initiated by several drugs and chemicals. Morphine, curare, chlorpromazine, concanavalin A, dextran, calcium ionophores and 48/80 are among the many agents in this category. Histamine secretion by non-immunological means, therefore, presents a serious health hazard to those who are exposed to such drugs for therapeutic reasons. Mast cells are also implicated in the inflammatory reactions associated with photoallergic and phototoxic dermatitis. In addition, they may also exacerbate the conjunctivitis and pulmonary distress caused by certain xenobiotics in the atmosphere. With the aid of biophysical techniques and compound 48/80, we are studying the molecular mechanism of histamine secretion. These studies may lead to the development of a safe method of control over both immunologically and environmentally related adverse conditions due to histamine secretion by mast cells.

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Ortner, M.J. and Chignell, C.F.: Spectroscopic studies of rat mast cells, mouse mastocytoma cells and compound 48/80. I. A spin label study of membrane fluidities and the effect of 48/80. Biochem. Pharmacol. 30: 277-282, 1981.

Ortner, M.J. and Chignell, C.F.: Spectroscopic studies of rat mast cells, mouse mastocytoma cells and compound 48/80. II. The synthesis and some binding properties of spin labeled 48/80. Biochem. Pharmacol. 30: 283-288, 1981.

Ortner, M.J. and Chignell, C.F.: Spectroscopic studies of rat mast cells, mouse mastocytoma cells and compound 48/80. III. Evidence for a protein binding site for compound 48/80. Biochem. Pharm. In Press.

Ortner, M.J. and Chignell, C.F.: The effects of concentration on the binding of compound 48/80 to rat mast cells: a fluorescence microscopy study. Immunopharmacology. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50032-04 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Binding of Copper to Human Serum Albumin

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

PI:	P. Mohanakrishnan	Visiting Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The binding of copper (Cu^{2+}) and nickel (Ni^{2+}) ions to serum albumin has been studied by ^{35}Cl nuclear magnetic resonance (NMR). The number of high affinity binding sites estimated by the NMR technique agreed well with previous data obtained with an ion-specific electrode. The estimated correlation time of albumin bound Ni^{2+} suggest that the N-terminal metal ion binding site undergoes more rapid motion than the protein molecule as a whole. Circular dichroism studies showed marked differences in Cu^{2+} and Ni^{2+} binding to dog serum albumin as compared to human and bovine serum albumin. Attempts to use ^{19}F NMR to monitor ligand binding to human serum albumin were unsuccessful.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fluorescence and circular dichroism techniques were used to study the binding of small molecules to HSA. Specifically labeled HSA was synthesized. Proton, carbon-13 and ^{19}FNM spectra of HSA and specifically labeled HSA were observed.

MAJOR FINDINGS AND PROPOSED COURSE: The binding of Cu^{2+} and Ni^{2+} to bovine, human and dog serum albumins was studied by ^{35}Cl NMR probe technique. The study indicates two different types of binding sites. For nickel, the number of relatively "high" affinity sites estimated are 1 to 2 for human, 2-3 for bovin and above 6 for dog serum albumin. The estimated rotational correlation time for chloride ions at the metal site were estimated to be of the order of picosecond. There is good agreement for the number of "high" affinity Ni^{2+} and Cu^{2+} sites for dog serum albumin. The initial irregular change of line width made it impossible for a similar comparison for the other two albumins. The estimated correlation times probably reflects a faster motion at the N-terminal site rather than albumin as a whole. The No of Ni^{2+} sites found from ^{35}Cl probe studies are in reasonable agreement with the No of Cu^{2+} sites found using ion selective electrode.

Cu^{2+} and Ni^{2+} binding to dog serum albumin was studied by circular dichroism. In the case of Cu^{2+}, the CD absorptions observed below 600 nm for bovine and human serum albumins were absent for dog serum albumin. Unlike, in the case of bovine and human albumins, nickel carbonate (in carbonate buffer pH 10.3) precipitated with dog serum albumins. The tendency for chelation is of the order BSA ~ HSA> CO_3^{2-} > dog serum albumin. These results are in agreement with other previous studies.

The lone sulfhydryl group of HSA was labeled with dansyl aminoethyl and iodoacetyl aminoethyl amino napthalene sulfonyl groups. The labeled protein was studied using fluorescence and induced dichroism. The fluorescence of the bound label was quenched more than 50% by the first equivalent of bilirubin and to about 10% by the second equivalent. Lifetime measurements will be carried out with the labeled protein in the presence and absence of small molecules. Critical distance measurements will be carried out from the label to the lone trp and fatty acid and bilirubin binding sites. The induced dichroism was found to be affected to a small amount by an initial addition of fatty acids. From the fatty acid binding studies, the dansyl probe seems to be in a more chiral environment than the ANS-type probe used.

HSA was labeled at the aspirin acetylation site with 2,6-dinitrs-4-trifluoromethyl benzene sulfonyl group and ^{19}FNMR spectrum of the labeled protein was observed. Studies with fluorosalicylic acid and dilfunisal were met with little success because of the instrumental limitations in partial proton decoupling. Alternatively HSA will be labeled with trifluoroacetyl group at the aspirin acetylation site and binding studies of the labeled proteins will be carri[illegible] with [illegible]ates using ^{19}FNMR. A tetrapeptide, Asp-Ala-His-Lys-OMe, with the sequence same as in N-terminal copper site was synthesized. Currently, experiments are being carried out to characterize the tetrapeptide and to study the binding of Cu^{2+} and Ni^{2+} as a model to N-terminal Cu^{2+} site. Proton NMR spectra of HSA at 400 MHz show

more than 12 C-2 and C_4-H protons of the His residues. Further studies will be carried out to make spectral assignments to individual His residues.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Copper and nickel are implied in several pathological and toxicological conditions. HSA is the major carrier of metal ions and small molecules in the serum. It is hence of tremendous significance in studying HSA and its metal ion and drug binding to unravel the underlying biological processes/mechanisms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50034-04 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Mechanism of the Oxygen-Insensitive Activation of 4-Nitroquinoline-1 Oxide

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

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
OTHERS:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

NONE

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

With one exception all mammalian nitroreductases are markedly inhibited by oxygen. Previous investigations have found that the oxygen-insensitive nitroreductase of rat liver supernatant is DT diaphorase. The reduction of 4-nitroquinoline-1-oxide (4-NQO) by supernatant was inhibited by oxygen only 10%, whereas dicoumarol inhibited 94%. The microsomal reduction of 4-NQO is oxygen sensitive as is characteristic of the usual nitroreductases. Several lines of evidence suggest that the oxygen inhibition of nitroreductases is the result of the air oxidation of the first reduction intermediate, the nitroaromatic anion free radical (RNO_2^-). $RNO_2^- + O_2 \longrightarrow RNO_2^- + O_2^-$

In view of the known rapid rate of the air oxidation of nitro anion free radicals ($k \geq 2.5 \times 10^5\ M^{-1}$), this oxygen-insensitive nitroreductase must not form the RNO_2^- or; at the very least, the radical must remain bound to the DT diaphorase and not be accessible to O_2. Presumably in this case, the first reduction intermediate formed is the corresponding nitroso compound, the two-electron reduction product.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance was used to search for the anion free radical metabolite of 4-nitroquinoline-1-oxide (4-NQO). Oxygen uptake and the rate of NADPH oxidation where determined using standard techniques. The adrenochrome assay used for superoxide is a modification of the method of Misra and Fridovich.

MAJOR FINDINGS AND PROPOSED COURSE: If the mechanism of microsomal nitroreduction of 4-NQO is like that of other oxygen-sensitive nitroreductases, then anaerobic incubations of microsomes, 4-NQO and NADPH should form the anion free radical, which should be detectable by electron spin resonance. Under aerobic conditions the free radical should not be detectable, but 4-NQO should stimulate O_2 uptake, superoxide formation, and NADPH oxidation by the microsomal incubation via the cyclic formation of the nitro anion free radical.

In contrast, we expect that incubations containing an equal amount of supernatant nitroreductase activity will not form the 4-NQO anion free radical, as determined by electron spin resonance. Again, in contrast to the microsomal incubations, the supernatant incubation should not consume more oxygen or oxidize more NADPH in the presence of 4-NQO.

Lastly, supernatant incubations should not form more superoxide in the presence of 4-NQO, whereas microsomal incubations are expected to generate more superoxide. Since the nitro compound-catalyzed oxygen consumption, superoxide formation and NADPH oxidation are thought to be due to the rapid air oxidation of RNO_2, these results would support the electron spin resonance results concerning the formation of the 4-NQO anion radical in the microsomal and supernatant incubations.

The 4-NQO radical anion was very difficult to detect even in microsomal incubations. The spectrum was very broad, and was typical of an immobilized free radical. This factor contributed to the difficulty in observing this species. The 4-NQO radical anion must be bound to the microsomes, and not free rotating in the buffer as are other nitro anion free radicals which we have investigated. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Either the nitroso or hydroxylamine metabolites of 4-NQO are thought to be the proximate carcinogen of 4-NQO. The rapid cellular reduction of 4-NQO in air is unique among nitro compounds and is thought responsible for its greater toxicity and mutagenicity and for its potent carcinogenicity relative to that of other nitro compounds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50038-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwave Radiation on the Cardiovascular System

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

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Melvyn Lieberman	Consultant	LEB	Duke University

COOPERATING UNITS (if any)

Physiology Department, Duke University

LAB/BRANCH
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 (200 words or less - underline keywords)

The objectives of this project are to determine the influence of microwave radiation on cardiac tissue in vitro and in vivo. A method for exposing isolated rat atria to microwave radiation has been developed. Preliminary data suggested 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. Also, certain biochemical and physiological parameters, which are indicative of cardiac function, have been measured in unanesthetized rats during whole body ventral exposure to 2450 MHz CW microwaves. Preliminary data suggest microwave exposure of 10 mw/cm^2 for 4 hr has no effect on heart rate, mean arterial blood pressure or colonic temperature.

PROJECT DESCRIPTION

METHODS EMPLOYED: a. Isolated rat atria maintained at either 22° or 37°C were exposed to microwave radiation at specific absorption rates of 2, 10 and 100 mW/g for periods up to 1.5 hours. During exposure, contraction rate and contractile force were monitored. For each experiment 2 pairs of atria were used, one control and one exposed, which were placed in specially designed tubes located in a waveguide exposure apparatus. In addition, the response of the tissue to drugs will be determined during microwave exposure.

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, electrocardiogram) and biochemical parameters were measured during 4 hour microwave exposure. The temperature humidity and noise level in the exposure chamber were maintained at 23°C, 60% and 70 dB respectively during the experimental period.

MAJOR FINDINGS AND PROPOSED COURSE: a. Preliminary data indicate that the exposure rates used (2 and 10 mw/g) has no overt effect on the rate or force of contraction of isolated atria at either incubation temperature. At 22°C the rate of contraction was 120 beats per minutes for both the control and exposed atria. Atria maintained at 37°C had a rate of contraction of 215 beats per minute, and was also unchanged by microwave exposure. These experiments will be completed and subsequently the response of atria to drugs will be determined during microwave exposure.

b. The data given for this experiment is the result of preliminary experiments and may be different from the final results obtained at the completion of the study. In the sham exposed rats the mean aterial blood pressure (MABP), heart rate (HR) and colonic temperature were 120 ± 10 mmHg, 300 ± 30 BPM, and 37.5 ± 0.8°C respectively. Four hour exposure to 10 mW/cm^2 microwave radiation had no influence on these parameters. In addition, a number of hematologic parameters were examined during the exposure period. No differences between the sham (0 mW/cm^2) or exposed (10 mW/cm^2) groups were noted for white and red cell numbers hematocrit, or plasma protein. This study will be computed in the coming year. The next study will examine the influence of microwave radiation on the biochemical and cardiovascular sequclae 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 cardiovascular system can be evaluated more effectively. This research on the effects of microwaves on the cardiovascular system is directed toward the mission of NIEHS to determine the health effects of physical factors in the environment.

PUBLICATION

Galvin, M.J., McRee and M. Lieberman. Effect of 2.45 GHz Microwave Radiation on Embryonic Quail Hearts. Bioelectromagentics 1(4): 389-396, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50039-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of 2450 MHz Microwave Radiation on Mast Cells

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

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation Workgroups

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The effect of microwave radiation was studied on active secretory cells. Rat peritoneal mast cells were exposed to 2450 MHz microwave radiation at specific absorption rates (SAR) of 8.5 and 42.5 mW/ml for periods of up to 3 hrs. Cells were maintained throughout exposure at 37°C. There was no effect on cell viability or spontaneous histamine release. Mast cells exposed to compound 48/80 after prior irradiation or during simultaneous irradiation secreted histamine in a manner similar to unexposed cells. In addition, mast cells exposed to concanavalin A or A23187 during simultaneous irradiation secreted histamine in a manner similar to unexposed cells. These studies are being extended to determine the influence of whole body exposure of rats to microwaves or mast cell physiology.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat peritoneal mast cells were obtained by peritoneal cavity lavage. The cells were irradiated in a waveguide exposure system using a frequency of 2450 MHz. Dosimetry in the cell chamber was determined directly from the time-temperature profiles to be 8.5 and 42.5 mW/ml respectively. The temperature during exposure was maintained at 37°C using a circulating water bath. Control cells were incubated 9.5 cm away from the microwave source and were not exposed to the radiation. After irradiation, samples of cells were removed and added to either Lockes solution or compound 48/80. In addition, cells were treated with concanavalin A, and A23187 during microwave exposure.
The drug-induced histamine release was terminated by addition of antimycin to the cell suspensions.

MAJOR FINDINGS: Mast cells were irradiated in a wave guide microwave exposure chamber at 2450 MHz with power absorptions of 8.2 and 41.0 mW/g for periods up to 3 hours. These levels of microwave absorption caused no change in the morphological characteristics or viability of the cells. Irradiated mast cells were stimulated with compound 48/80, a potent, non-cytotoxic histamine releasing agent. The dose response curves showed that neither prior nor simultaneous irradiation of mast cells at 37°C affected 48/80 induced secretion. When irradiated mast cells were treated with Con A (25 or 100 µg/ml) or A23187 (0.25 or 1.0 µM), neither of the microwave dosages influenced the release of histamine. These data suggest that microwave radiation at the frequency and absorption rates used in these studies has no influence on histamine secretion evoked by 48/80, calcium influx of lectin binding with cells are maintained at 37°C.

Mast cells irradiated at 44.0°C responded to 48/80 in a manner similar to identical cells heated conventionally to 44.4°C. In contrast, cells irradiated at 43.5°C. were unchanged from cells heated conventionally to 43.5°C. This indicates that the SAR used (41.0mW/ml) did not heat the cells more than 0.9°C above ambient levels, and that the irradiation procedure may have heated the cells 0.4°C above ambient.

These studies will be extended to examine the influence of whole body microwave exposure on peritoneal mast cells. In addition, rats which have been depleted of mast cells by 48/80 injections will be exposed to microwaves during mast cell regeneration. These two additional studies will provide needed information on the interaction of microwaves with cells and body systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough study of the effects of microwave radiation on all biological levels must be completed before microwave radiation exposure can be considered safe. Waveguide systems have been developed to study the effects of nonionizing radiation on cell membranes. The complicated series of events resulting in mast cell membrane fusion and histamine secretion are unimpaired by the dosage and frequency of microwave radiation used in these experiments. Studies are being planned for assessing the effects of whole body microwave irradiation on mast cell function in rats. By using in vivo and in vitro techniques microwave interaction with cells can be emulated more effectively. This research on the effects of microwaves on the cardiovascular system is directed toward the mission of NIEHS to determine the health effect of physical factors in the environment.

PROPOSED COURSE: This project has been completed.

PUBLICATIONS

Ortner, M.J., and M.J. Galvin: The effect of 2450 MHz microwave radiation on histamine secretion by rat peritoneal mast cells. Cell Biophysics, 2, 127 (1980).

Galvin, M.J. and M.J. Ortner: Effects of 2450 MHzmicrowave radiation on concanavalin A or inophore induced histamine release from rat peritoneal mast cells. Int. J. Rad. Res. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50040-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Influence of 2450 MHz Microwave Radiation on Cats subjected to Myocardial Ischemia

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

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

None

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 (200 words or less - underline keywords)

The influence of direct microwave irradiation (2.45 GHz, CW) of the intact exposed heart on cardiac function in cats with and without myocardial ischemia is being examined. Myocardial ischemia (MI) is induced by occlusion of the left anterior descending coronary artery. In the sham and sham-plus-microwave exposed animals the coronary artery is isolated but not occluded. The exposed hearts are irradiated at a specific absorption rate of 30 mW/g or sham irradiated and are monitored for 5 hours. Mean arterial blood pressure (MABP), cardiac output (CO), heart rate (HR), plasma and myocardial creatine phosphokinase (CPK), and S-T segment are not influenced by microwave irradiation of the myocardium. Furthermore, in the MI-plus-microwaves and MI cats, comparable values of MABP (124 vs 114 mmHg), (128 vs 130 ml/min kg), HR (119 vs 124 beats/min), plasma CPK (8.5 vs 9.0 IU/mg protein) and S-T segment (0.60 vs 0.63 mV) are observed at 5 hr post occlusion. Thus, the results suggest that 5-hour exposure of the cat myocardium to microwave radiation has no effect on the parameters examined. In addition, this environmental factor has no influence on the course of acute myocardial ischemia in cats.

PROJECT DESCRIPTION

METHODS EMPLOYED: This study examines the effects of direct microwave radiation of the heart on cardiac function and the influence of irradiation on the course of acute myocardial ischemia in cats. Cats are subjected to either myocardial ischemia by occlusion of the left anterior descending coronary artery or a sham operation. The myocardium is exposed to 2450 MHz microwave radiation at 30 mW/g tissue, and certain physiological and biochemical indices are monitored for five hours post occlusion. Appropriate control cats are also included in this study. Serial blood samples are taken every hour during the course of the experiment and at termination the myocardium is biopsied. Tissues were examined for alterations in cellular integrity utilizing biochemical techniques. Plasma creatine phosphokinase activity, S-T segment of the electrocardiogram, arterial blood pressure, cardiac output, and heart rate are monitored during the 5-hour microwave exposure.

MAJOR FINDINGS AND PROPOSED COURSE: During the past few months a model has been developed to examine the interactions of 2450 MHz CW radiation on tissues which are ischemic. The model in this study is designed to examine the possible interactions between microwave radiation, and normal and ischemic cardiac cells. Mean arterial blood pressure (MABP), cardiac output (CO), heart rate (HR), plasma and myocardial creatine phosphokinase (CPK), and S-T segment are not influenced by microwave irradiation of the myocardium. Furthermore, in the MIplus-microwaves and MI cats, comparable values of MABP (124 vs 114 mmHg), (128 vs 130 ml/min kg), HR (119 vs 124 beats/min), plasma CPK (8.5 vs 9.0 IU/mg protein) and S-T segment (0.60 vs. 0.63 mV) are observed at 5hr post occlusion. Thus, 5-hour exposure of the cat myocardium to microwave radiation has no effect on the parameters examined, and in addition, this environmental factor has no influence on the course of acute myocardial ischemia in cats. These studies are being expanded to include an examination of the response of unanesthetized animals to whole body microwave radiation. See project Z01 ES 50038-02 LEB, which details the plans for these studies.

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 research on the effects of microwaves on ischemic injury is directed toward the mission of NIEHS to determine the health effects of physical factors in the environment. The effects of 2450 MHz microwaves may not be evident in normal tissue, but in ischemic tissue there may be influences from nonionizing radiation. The use of cells which have been compromised may be a useful system for evaluation of possible microwave effects. This study is complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50041-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Permeability of the Cochlear Partition Assessed by Electrochemical Potential change during Anoxia

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Ionic permeability changes in the endolymph-perilymph barrier are significant factors in the physiological mechanisms underlying noise induced hearing loss. The purpose of this study is to estimate membrane permeability, K^+ in the normal and pathological cochlea by measuring alterations of the passive K^+ efflux from the endolymph and the electrochemical potential difference between the endolymph and perilymph during permanent anoxia.

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs anesthetized with sodium pentobarbital were used. The endocochlear potential (EP) and K^+ concentrations of the endolymph and perilymph were simultaneously measured in the basal turn of the cochlea with a pair of double barreled K^+ selective liquid membrane electrodes. When the active transport is abolished by anoxia, K^+ ions diffuse passively from the endolymph to perilymph. If the volume of the cochlear endolymph is assumed to remain unchanged during anoxia, the passive flow of K^+ ions from the endolymph can be expressed by

$$J_K^{diffusion} = V_{end} \frac{d[K^+_{end}]}{dt}$$

The K^+ conductance of the endolymph-perilymph barrier, G_K can be computed by

$$G_K = \frac{V_{end} F \frac{[K^+_{end}]}{dt}}{\frac{RT}{F} \ln \frac{[K^+_{end}]}{[K^+_{peri}]}} + \Delta\psi$$

The relationship between the permeability coefficient, P_K and G_K is given by

$$P_K = \frac{RT}{F^2} \cdot \frac{G_K}{\overline{[K^+]}}$$

where

$$\overline{[K^+]} = \frac{\Delta[K^+_{end-peri}]}{\ln \frac{[K^+_{end}]}{[K^+_{peri}]}}$$

Three groups of guinea pigs were used: one group of guinea pigs was treated with kanamycin 400 mg/kg for 3 weeks and tested 2 weeks after treatment. A second group was exposed to broad band noise at 115 dBA for 11 to 15 days. A third group was used as control animals.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potentials. The CM sensitivity (expressed by sound intensity required to elicit 100 μv CM) was reduced to 88.8 ± 5.3 dB SPL and the maximum CM output was suppressed to 270 ± 90 μV in noise exposed guinea pigs. The CM max in all kanamycin-treated guinea pigs was less than 80 μV.

2. K^+ conductance and K^+ permeability constant of the endolymph-perilymph barrier. The preanoxic values of K^+ electrochemical potential difference between endolymph and perilymph were 189.6 ± 4.9 mV, 190.4 ± 5.9 mV and 202.2 ± 6.2 mV in [illegible] noise exposed and kanamycin-treated guinea pigs respectively. The rate of decline of the electrochemical potential difference during anoxia was slower in noise exposed guinea pigs and kanamycin treated tuinea pigs than control animals. In control guinea pigs, the mean rate of fall of $[K^+_{end}]$ decreased with time and

reached the minimum value (250 μM/min) 30 min after anoxia. In noise exposed guinea pigs the time course was similar to that observed in control animals, but in kanamycin treated guinea pigs the rate of fall of $[K^+_{end}]$ was markedly reduced. The mean G_K value averaged from 10 to 30 min after onset of anoxia was (34.85 ± 5.60) x 10^{-6} mho in normal guinea pigs, whereas the mean G_K averaged during the same period was (20.43 ± 2.53) x 10^{-6} mho in noise exposed guinea pigs and (8.13 ± 1.82) x 10^{-6} mho in kanamycin treated guinea pigs.

The P_K values of the endolymph-perilymph barrier averaged from 10 to 30 min after anoxia were (112.33 ± 16.98) x 10^{-9} cm^3sec^{-1} in kanamycin treated guinea pigs. These values were considerably lower than that of (193.62 ± 34.81) x 10^{-9} cm^3sec^{-1} of observed in control guinea pigs.

The mean values of G_K and P_K were compared with those computed from the rate constant for K^+, λ_K.

$$G_K = \frac{F^2 . \lambda_K V_{end} [K_{end}]}{\Delta\tilde{\mu}_K}$$

where $\Delta\tilde{\mu}_K$ is electrochemical potential difference between endolymph and perilymph.

The values of G_K derived from the rate constant in normal and noise exposed guinea pigs were calculated to be 34.46 x 10^{-6} mho and 16.77 x 10^{-6} mho respectively.

A manuscript has been published and this project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is impossible at present to measure the permeability of the various cochlear membranes and one useful approach is to determine the diffusional alterations in the endolymphatic ion concentration during anoxia. The present study demonstrates that the magnitude of decrease of K permeability of the endolymph-perilymph barrier by noise or kanamycin is corrleated with suppresion of hair cell function. The results obtained will shed light on the physiological mechanisms of noise or drug induced ear damage.

PUBLICATIONS

Konishi, T. and Salt, A.N.: Permeability to potassium of the endolymph-perilymph barrier and its possible relation to hair cell function. Exp. Brain Res. 40: 457-463, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50042-03 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comparison of Impact Noise and Continuous Noise Effects on Cochlear Function

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS
	Hirohiko Mori	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 early electrophysiological changes occuring during exposure to energy equivalent impact or continuous noise exposure in guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Impact noise with peak sound pressure of 132 dB SPL and a B duration of 29 msec was generated by a mechanical impact noise generator. Continuous broad band noise of equal energy to a given intensity of impact noise was generated by a loud speaker system. Conscious guinea pigs with implanted round window electrodes, or anesthetized guinea pigs with differential electrodes placed in the perilymphatic space, were exposed to a 20 min period of impact noise or broad band noise of equal energy. The suppression of tone induced responses (cochlear microphonics and action potentials) during and 1 hour following noise exposure was monitored by presenting 6 kHz test tones at 72 dB SPL.

MAJOR FINDINGS AND PROPOSED COURSE: When anesthetized guinea pigs were exposed to 132 dB peak SPL impact noise at 1.2 impacts/sec (Leq-lin level of 105 dB) for 20 mins the cochlear microphonics (CM) and action potentials (AP) were suppressed to 24.0% and 49.5% of their pre-exposure values respectively. During 1 hour recovery the CM and AP increased to 88.9% and 101.4% of their pre-exposure magnitudes respectively. In anesthetized guinea pigs exposed to 105 dB SPL continuous noise for 20 mins the CM was reduced to 80.5% of the pre-exposure level and in all cases the AP was totally abolished. During 1 hour recovery the CM and AP increased to 106.0% and 61.4% of their pre-exposure values respectively. Similar results were found when guinea pigs with chronically implanted round window electrodes were exposed to continuous or impact noise.

Our results indicate that during noise exposure and for 1 hour following the exposure the degree of response suppression produced by impact and continuous noise of equal energy is not equivalent. It is not possible to infer from these data whether the differences would persist during chronic, long term exposure. A pneumatically driven impact noise generator has been developed to improve durability. The measurements of acoustic characteristics was recently completed.

Using a pneumatically driven impact noise generator, effects of exposure to impact or continuous noise of long duration will be compared with those found in short term experiments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The equal energy hypothesis has been widely employed in the development of European noise regulations. Our results indicate that the measurement of energy may not be adequate to predict hearing loss in situations where impact noise is present. Further study of the relationship between hearing loss and the parameters of impact noise exposure are essential for the development of adequate noise regulations.

PUBLICATIONS

Salt, A.N., Konishi, T., Cook, R.O. and Akay, A.: Comparison between the effects of continuous and impact noise on cochlear potentials in guinea pigs. J. Acoust. Soc. Amer. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50043-03 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Noise on Embryo/Fetal Development in the Guinea Pig

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

PI:	Peter Nawrot	Visiting Associate	LEB	NIEHS
	Reginald Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.50	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

All laboratory studies on teratogenic potential of noise reported to date, have used the rat or mouse as the experimental animal. Yet the guinea pig is probably a more suitable experimental animal for such studies. In addition to endocrine similarity to man, the audibility curve for the guinea pig is probably closer to that of the human than any other mammal except primates and chinchillas. The auditory sensitivity of rats or mice is such that the most sensitive frequency is nearly a decade above that of humans. While the guinea pig has been extensively used to investigate the effects of noise on the inner ear and on basic mechanisms of hearing the teratogenic potential of noise in the guinea pig has not been assessed.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Weaving room noise was selected as the exposure agent since it was utilized in the only study on the effects of noise on the guinea pig fetus and since it is experienced at high levels (105 dBA) by a significant number of women worldwide (in the hundred of thousands), and is a relatively broad band steady state noise which does not vary greatly worldwide. Mated females will be assigned randomly to one of four experimental groups; i.e. to a "Noise" group or to a "Control". Each group will contain at least 20 pregnant females. The guinea pigs in the "Noise" groups will be housed in an IAC animal exposure chamber from days 1 through 11 or from days 11 through 34 of gestation (postimplantation exposure). The days of exposure (1 through 11 and 11 through 34 of gestation) were chosen because implantation in the guinea pig takes place on day 6, but the connection with the uterine lumen is not lost until day 11 of gestation. Continuous weaving room noise will be presented at an intensity of 115 dBA for 8 hours daily. During the quiet period, the animals will be housed in a different chamber without the noise stimulus.

Body weights of all mated animals will be taken on days 1, 11, 34 and 35 of gestation. On day 35 of gestation, the guinea pigs will be coded before being transported to RTI where they will be sacrificed by CO_2 inhalation. Their reproductive status will be determined, the fetuses will be counted and then each will be examined for external, visceral and skeletal alterations.

The guinea pigs will be exposed to noise in an IAC animal exposure chamber modified for optimum sound field uniformity. The chamber provides ample protection from sound penetration through the walls. The data will be analyzed by the Mann-Whitney U-test with the litter considered as the experimental unit. A 5% probability level will be accepted.

MAJOR FINDINGS AND PROPOSED COURSE: The special acoustic chamber needed to efficiently carry out this experiment has been designed and ordered. The experiment will begin after the chamber is equipped with a process which has been subject to unanticipated procurement delays. This project has been discontinued due to the departure from the Institute of one of the Principle Investigators.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE INSTITUTE: Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been expressed regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. The ability to predict risk factors for human exposure is to some degree related to the occurance of similar effects in different species and to the occurance of effects in species whose auditory systems more closely resemble those of humans. Use of guinea pigs rather than rats or mice satisfies both criteria.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50045-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Relationship of Catecholamine Levels and Fetolethality in Noise Exposed Mice

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

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
	Peter Nawrot	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, P. O. Box 12233, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Since a previous experiment revealed that late stage pregnancy exposure of CF-1 mice to high frequency noise resulted in a significant increase in fetolethality, the hormonal/biochemical correlates of this effect were sought. Since corticosterone levels were measured in a previous experiment (see Z01 ES 50044-02 LEB) and found to be unaffected by noise exposure, this experiment focused on catecholamines, Exogenous introduction of these substances has been found to increase fetolethality. Exposure period was 12 hours (noon to midnight) and noise was a swept band at 18-20 KHz. Reproductive-teratogenic effects determined by standard techniques included lower maternal and fetal weights increased entire litter resorption, and a significant increase in the total number of malformed fetuses. A suggestive increase in plasma epinepherine levels was noted ($p < 0.06$) and a significant increase in plasma norepinepherine levels was associated with late stage exposure. Uterine norepinepherine levels were not affected when measured on days 1 and 6 of gestation but were significantly elevated when measured on days 15.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Noise exposure: Mated mice assigned to the noise exposed groups were exposed from noon to midnight to a cycled high frequency tone which swept from 18 KHz to 20 KHz in 3 seconds then repeated the process after a 100 msec delay. The noise source was a device commerically marketed for repelling feral rodents. A completed description of the sound production and monitoring system and of the spectral characteristics of the sound is contained in an earlier report. There were two noise exposed groups. One group was exposed from day 1-6 of gestation and a second group was exposed from day 6-15. Both groups were housed in one chamber from day 1-18 of gestation or until sacrificed.

Teratology study: On day 18 of gestation the dams from each group (control, noise exposed days on 1-6, or days 6-15) involved in the teratogenicity study were killed by cervical dislocation and their reproductive status was noted. The conceptus at each site was examined and classified as being resorbed, dead or alive. The uteri of females designated as non-pregnant on the basis of visual examination were immersed in a 10% solution of ammonium sulfide for at least 10 minutes to identify early entire litter resorptions. The full-termed fetuses (live and dead) were sexed, weighed, and examined for external malformations. Any fetus weighing less than 0.5g or less than two-thirds the average of the larger litter mates were termed stunted. At least one-third of the fetuses of each litter were examined for visceral alterations. In addition, all stunted fetuses, and those with external malformations were examined for visceral alterations. The heads of the fetuses examined for visceral alterations were severed, fixed in Bouin's solution, and subsequently were examined by the free-hand razor section technique. All live and dead fetuses were cleared with KOH, stained with Alizarin Red and examined for skeletal alterations.

Catecholamine study: Blood and uterine samples were obtained on days 1 and 6 of gestation from the group exposed to noise on days 1-6; and on days 6, 10 and 15 of gestation from the group exposed to noise on days 6-15; and on days 1, 6, 10 and 15 of gestation from the control group. One each collection day blood and uterine samples were obtained at 3 invariant times (12:15 PM, 2:15 PM, and 4:15 PM). For the noise exposed groups, these collection times occurred 15 minutes, 135 minutes, and 255 minutes after initiation of noise exposure on day of collection. In the collection procedure, mice were anesthesized by intraperitoneal injection of 3 ml/kg pentobarbital sodium and the blood samples were taken after incision from the subclavian arteries directly into cold heparimized collecting tubes. To prevent oxidation of catecholamines, blood was immediately mixed with reduced gluthathione (approximately 2 mg of gluthathione per 1 ml of blood) and the tubes placed in an ice bath. The blood samples were centrifuged at 4°C to separate plasma from cells. The plasma was removed, placed in a screw-top vials, frozen on dry ice, then stored at -80°C until assayed for catecholamines. Immediately after blood sample collection the uterus was dissected, opened, and implantation sites removed. The uterine tissues were place in screw-top vials and frozen on dry ice for subsequent determination of catecholamine level. Blood and uterine samples were retained only from females determined to be pregnant.

Uterine catecholamine levels were measured by an HPLC method based on modifications of a technique described by Keller et al. (1976). Modification consisted

of the use of pre-extracted (with alumina) plasma as standard curve vehicle. The zero corrected standard curve using this modification was accurate at ± 20 picograms wherease when acid or water alone was used, accuracy was on the order of 200 picograms.

Plasma catecholamine levels were measured twice for each sample; once by the HPLC method described above and once by a radioenzymatic method modified.

MAJOR FINDINGS AND PROPOSED COURSE: Although the late stage of fetolethality response of the CF-1 mouse to this noise paradigm was not duplicated by the response of the CD-1 mouse, the latter demonstrated a significant increase in the total number of malformations in addition to the early stage resorption, slightly reduced pregnancy maintainence, and reduced fetal and maternal weight responses noted in most other experiments in this series. Of nine groups of pregnant mice exposed in experiments spanning five years, pregnancy maintainence was lower in all noise exposed groups relative to controls, while teratogenic responses and late stage fetolethality occurred only once, suggesting that interference with pregnancy maintainence is a highly probable result of noise stress while overt teratogenicity or late stage fetolethality require synergism with with some unknown, possibly genetic, factor.

Although this experiment is apparently the first in which catecholamine levels have been measured in the plasma and uteri of pregnant mice, the course of uterine norepinepherine (NE) levels in controls was similar to that observed in other species (rats and guinea pigs). Noise exposure corresponded to failure of the normally rapid reduction in uterine NE levels during the last half of gestation. The significance of this development cannot be assessed from this experiment, but it is interesting to note that the normal rise in plasma corticosterone between days 10-15 corresponds to the same period in which uterine NE levels are normally in rapid decline. Experiments where catecholamines have been exogenously applied have shown that these substances interfere with implantation possibly through tubal motility changes, and with the conceptus after implantation with effects ranging from reduced fetal weights at near physiological levels to fetal death at high levels. As a result, future experiments should investigate the effect of more precisely timed noise applications immediately preceding implantation, and during very late stage pregnancy (days 15-18). The latter has not been investigated during noise experiments.

SIGNIFICANCE TO BIOLOGICAL RESEARCH AND PROGRAMS OF THE INSTITUTE: Identification of the hormonial/biochemical correlates of the non auditory effects of noise is a necessary step toward understanding these effects sufficiently for predictive purposes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50046-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanisms of Chemically Induced Photosensitivity

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

PI:	Colin F. Chignell	Chief	LEB NIEHS
OTHERS:	Ann Motten	NRS Postdoctoral Fellow	LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	1.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this study is to determine the role played by light induced free radicals in chemically induced skin photosensitivity. Light irradiation of 4-aminobenzenesulfonamide (sulfanilamide) resulted in the production of the following radicals: $^{\bullet}NH_2$, 4-$\dot{N}HC_6H_4SO_2NH_2$, $H^{\bullet}$, 4-$H_2NO_2SC_6H_4$, SO_2NH_2, 4-$NH_2C_6H_4SO_2$, SO_3^-. Which radicals were produced depended on the pH of the solution and the wavelength of irradiating light. Under the same conditions, sulfacetamide, sulfadiazine, sulfathiazole, carbutamide, and tolbutamide each gave rise to several of the same radicals, notably $SO_3^{\bullet -}$. The generation of these free radical species may explain the phototoxic and photoallergic properties of these compounds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Many chemicals are known to cause photosensitivity in certain individuals. The photosensitive response may be one of two types, either phototoxic or photoallergic. The phototoxic reaction generally occurs during a subject's first exposure to light after the administration or topical application of a chemical and usually takes the form of an exaggerated erythemal response ("sunburn"). Photoallergic individuals may also exhibit an initial erythemal reaction. As this subsides, delayed abnormal responses begin to appear including papular, eczematous and urticarial reactions. Such reactions to light may persist for months after avoidance of the photoallergen.

While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanism of photoxicity and photoallergy is unknown. In this study, 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 substituted aromatic compounds. The structure of the trapped radicals, and the initiating free radicals has been determined with the aid of electron spin resonance. The fate of the photosensitizing compound in hairless mouse skin is also being studied using photoacoustic spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: In aqueous solution, sulfanilamide (4-aminobenzenesulfonamide, R = H) is photolyzed according to the following reaction scheme:

$$4\text{-}NH_2C_6H_4SO_2NHR \xrightarrow{h\nu} NH_2{+}\dot{C}_6H_4SO_2NHR \quad (1)$$

$$\xrightarrow{h\nu} 4\text{-}NH_2\dot{C}_6H_4{+}\dot{S}O_2NHR \quad (2)$$

$$\xrightarrow{h\nu} 4\text{-}NH_2C_6H_4\dot{S}O_2{+}\dot{N}HR \quad (3)$$

$$\xrightarrow{h\nu} 4\text{-}\dot{N}HC_6H_4SO_2NHR{+}H\cdot \quad (4)$$

The primary radicals produced also react further to give other radical and non-radical species.

$$2\ \ 4\text{-}NH_2C_6H_4\dot{S}O_2 \longrightarrow 4\text{-}NH_2C_6H_4SO_3^- + NH_2C_6H_4SO_2\text{-}$$

$$4\text{-}NH_2C_6H_4SO_3^- \xrightarrow{h\nu} 4\text{-}NH_2\dot{C}_6H_4{+}SO_3^{\cdot -}$$

$$2\ \dot{S}O_3^- \longrightarrow SO_3{+}SO_3^{2-}$$

$$SO_3{+}H_2O \longrightarrow 2H^+{+}SO_4^{2-}$$

Other compounds which have also been studied include sulfacetamide, sulfadiazine, sulfathiazole, carbutamide, and tolbutamide. These photosensitizers also give rise to free radical intermediates upon irradiation. The reaction pathways identified below have been observed.

Sulfacetamide	(R = $COCH_3$)	(1), (3), (4)
Sulfadiazine	(R = $C_4N_2H_3$)	(1), (4)
Carbutamide	(R = CONHBu)	(1), (3), (4)
Sulfathiazole	(R = C_3NSH_2)	$\dot{SO}_3^-$ only

Reaction (4) predominates when the anion form is present (basic pH) and can be excited using light of longer wavelength than that required for the other pathways.

Preliminary work has begun to determine the fate of these drugs in hairless mouse skin. Tetrachlorosalicylanilide, another photosensitizer appears to be bound or metabolized in the epidermis of freshly killed mice. The metabolism of photosensitizer after irradiation will also be followed in live mice.

Other compounds which have also been studied include sulfacetamide, sulfadiazine, sulfathiazole, tolbutamide, and carbutamide.

These studies are being extended to other aromatic molecules, including those found in effluents from energy-related processes, that are known to cause skin photosensitivity. Preliminary work has already begun on anthracene since this agent is both phototoxic and photocarcinogenic. Evidence is also being sought for other possible mechanisms of photosensitization including singlet oxygen formation ("photodynamic" effect) and energy transfer to biologically important macromolecules.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many drugs and other environmental chemcials are known to cause skin photosensitization. These studies will help to provide a molecular basis for understanding this toxic effect. When the mechanism of photosensitization is known it may be possible to design a test which will identify those chemicals which could have potentially harmful effects on the skin in the presence of light.

PUBLICATIONS

Chignell, C.F., Kalyanaraman, B., Mason, R.P. and Sik, R.H. Spectroscopic Studies of Cutaneous Photosensitizing Agents I. Spin trapping of photolysis products from sulfanilamide, 4-aminobenzoic acid and related compounds. Photochem. Photobiol. 32: 563-571, 1980.

Chignell, C.F., Kalyanaraman, B., Sik, R.H. and Mason, R.P.: Spectroscopic studies of cutaneous photosensitizing agents II Spin trapping of photolysis products from sulfanilamide and 4-aminobenzoic acid using 5,5-dimethyl-1-pyrroline-1-oxide (DMPO). Photochem. Photobiol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50047-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biological Effects of Fluorescent Lighting

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

PI:	Colin F. Chignell	Chief	LEB	NIEHS
OTHER:	Beth C. Gladen	Statistician	BB	NIEHS
	Donald B. Feldman	Veterinarian	RTI	RTP

COOPERATING UNITS (if any)

Biometry Branch, NIEHS
Research Triangle Institute, Research Triangle Park, N.C.

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this study is to determine whether artificial lights have any adverse biological effects. The effect of three different fluorescent lighting systems (simulated daylight, cool-white, and pink) on the C_3H mouse has been studied. The parameters measured include longevity, tumor incidence, reproduction and pathology. The median times for mammary tumor development were 51 weeks, 47 weeks and 42 weeks for female mice kept under the daylight, cool-white and pink fluorescent lights respectively. The first litters of mice kept under the cool-white and pink lights were signficiantly delayed when compared to mice housed under daylight simulating conditions. Finally, the dams in the cool-white group gave birth to smaller litters than did dams exposed to the daylight lights. The possible human health effects of these findings are at the present unclear.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purpose of this study is to determine the biological effects of fluorescent lighting on the C_3H mouse. This strain was selected because it spontaneously develops mammary tumors and because previous studies have suggested that exposure to certain fluorescent lighting decreases lifespan. Three types of fluorescent lights were selected for this work: cool white (GE F40CW), daylight (GE F40C50) and pink (GE F40PK). The lights were kept on for 12 hrs daily (6:00AM-6:00PM). Sexually mature female mice were allowed to conceive under the cool white light and then were transferred to the appropriate light environment for delivery. After weaning the mice were paired and kept under the same lighting conditions.

MAJOR FINDINGS AND PROPOSED COURSE: At the conclusion of this study, which ran for 19 months, 97% of the female mice in the daylight and cool-white groups and 100% of the females in the pink group had developed mammary tumors. The median times for mammary tumor development were 51 weeks, 47 weeks and 42 weeks for the daylight, cool-white and pink groups respectively. Histological examination of female mice from each group revealed that all cases were carcinoma or adenocarcinoma of the mammary gland.

The median age of the dams at first litter and the median time between the first and second litter were not significantly different when the three groups of mice were compared. However, there was some evidence that the first litters were significantly delayed in both the cool-white ($p=0.01$) and pink ($p=0.05$) groups compared to the daylight group. When the litter sizes were compared in chronological order, it was found that mice in the cool-white group gave birth to significantly smaller ($p=0.009$) litters (0.5 pups/litter) than the daylight group.

No statistically significant differences among the three groups were noted in neonatal mortality, pup weight at weaning, sex ratio, total number of litters/dam and total number of pups/dam.

This project has now been completed. No further studies are contemplated at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although fluorescent lights are more efficient than conventional incandescent lights, the energy spectrum of fluorescent lighting is considerably different from that of natural daylight. It, therefore, is important to know whether the distorted spectrum of fluorescent lights produces any undesirable biological effects.

PUBLICATIONS

Chignell, C.F., Sik, R.H., Gladen, B.C. and Feldman, D.B.: The effect of different types of fluorescent lighting on growth, reproduction and mammary tumor development in the C_3H mouse. Photochem. Photobiol. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50048-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of Lung Lamellar Bodies Using Spin Labels

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

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
	Gary E.R. Hook	Research Chemist	LPFT	NIEHS
	Judson Spalding	Research Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

An electron spin resonance probe (5-doxyl methyl stearate) has been used to characterize the fluidity of lung Type II cell lamellar bodies which store pulmonary surfactant. These lecithin containing multilaminate structures were considerably less rigid than erythrocyte ghosts or normal leukocyte membranes, and were not changed by freezing or sonication. The lamellar bodies showed a temperature-dependent fluidity profile which was identical to liposomes made from lamellar body - extracted lipids; however it differed significantly from liposomes made of pure dipalmitoyl lecithin (DPL). Furthermore, liposomes made from a DPL-phosphatidyl glycerol combination which closely resembled the major lamellar body phospholipid components, were significantly different from the natural lamellar body liposomes. This indicates that perhaps minor phospholipids may play an important role in determining the molecular order maintained within the lamellar bodies.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The properties of lamellar bodies from rabbit lung type II cells have been investigated using electron spin resonance (ESR) probes. The lamellar bodies were extracted and purified according to well established procedures. The spin label (5-doxyl methyl stearate) was purchased from Syva Associates, Palo Alto, California. ESR measurements were taken using a Varian E-109 X-band spectrometer equipped with a E-238 Tm_{110} cavity and a variable temperature apparatus.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Artificial Systems. The effect of temperature on the motion of 5-doxyl methyl stearate (5 DMS) in liposomes made from pure dipalmitoyl lecithin (DPL) was investigated. At high temperature (52°C - 41°C) the probe reported an almost constant fluidity; however between 41°C and 38°C a sharp transition point occurred. The increase in fluidity of the probe then progressed in a linear manner as far as 22°C. Liposomes made from egg phosphatidyl choline also showed a similar transition temperature; however the increase in fluidity as a function of temperature was much less than the DPL liposomes. We have also examined phosphatidyl glycerol ((PG) which constitutes about 12% of the lamellar body phospholipids) and DPL/PG combination liposomes. The PG liposomes bound the spin label in a more highly immobilized manner than the DPL liposomes at temperatures between 45°C-21°C and the combination liposomes were intermediate. 2. Lamellar Bodies. The effect of temperature on the motion of 5-DMS in extracted lamellar bodies was studied. The probe showed a fluidity in the lamellar bodies which was closer to the artificial systems and much higher than human erythrocyte ghosts or normal leukocyte membranes. The effect of temperature showed a slight transition point at 40°C and an additional transition at 28°C. Lamellar bodies which had been sonicated or frozen were similar to the native state organelle. In further experiments, mixed lipids were extracted from the lamellar bodies using chloroform/methanol (2:1), and liposomes were formed. The motion of the spin label in the liposomes was unchanged from that of the native state lamellar bodies at temperatures between 50°C-22°C.

Although the motion of the spin label in the lamellar bodies was closer to that of the artificial systems than that of ghost or leukocyte membranes, it was significantly different from the artificial liposomes composed of the major constituent phospholipids (DPL/PG). This finding indicates that perhaps a minor lipid component may be influencing the structure of the lamellar bodies.

These spin probe studies are being currently expanded to study other artificial systems including some of the minor lipid components of the lamellar bodies. We hope eventually to duplicate the natural fluidity profile and thereby determine the effect of minor components of the lamellar bodies. In addition, we are currently isolating sufficient quantities of lamellar bodies to isolate and separate the component lipids to determine the effect of each on the temperature profile of the interacalated spin label. When these studies are completed the effect of exposure to oxidizing gases (SOx, NOx) on the molecular structure of the lung lamellar bodies will be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The acellular layer of surfactant which is essential for normal pulmonary function is stored by the lamellar bodies of Type II cells. These lecithin-rich organelles also contain hydrolytic enzymes and may be a source of the hydrolases secreted into the acellular lining of the branchioles. Although the lipid components of these airways have been isolated and characterized, there has been no thorough study of the interaction of these components with one another within the lamellar body. This study should provide information leading to a more complete characterization of the molecular structure of this organelle which is essential to normal pulmonary function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50049-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Spin-trapping and Direct ESR Studies of Anticancer Quinone Metabolites

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

PI:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
OTHERS:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Spin-trapping of the superoxide free radical has provided evidence for the formation of this species in microsomal incubations of adriamycin, daunorubicin, and mitomycin C. The time sequence of the appearance of the spin-trapped superoxide and the semiquinone radical metabolite of these quinone-containing anticancer drugs indicates that air oxidation of the semiquinone metabolite is responsible for the superoxide formation. Superoxide dismutase prevents the formation of the superoxide spin adducts. Microsomal incubations containing DNA-intercalated anthracyclines produce much less superoxide than incubations without DNA. The first clear evidence for the semiquinone metabolite of mitomycin C in a biological system was obtained using electron spin resonance.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: ESR measurements were made with a Varian century series E109 spectrometer equipped with a TM_{110} cavity. g Value measurements were done with an E-232 dual sample cavity using Fremy's salt as a g-standard (g = 2.0055).

MAJOR FINDINGS AND PROPOSED COURSE: In our study we have unambiguously observed the mitomycin C semiquinone in anaerobic microsomal incubations and the spin-trapped superoxide in similar aerobic incubations. In addition, we report direct ESR and spin-trapping evidence suggesting that the autoxidation of the mitomycin C semiquinone and the anthracycline semiquinone free radicals, in fact does result in the formation of superoxide. The spin-trapped superoxide ESR spectrum is characterized by known hyperfine couplings, so the identity of this species is not dependent upon the activity of superoxide dismutase, as was the case in the earlier investigations.

At present two free radical mechanisms for the cytotoxic anticancer quinone compounds have been proposed. Evidence has been presented suggesting that the formation of the semiquinone is rate-determining in the covalent binding of mitomycin C to DNA, and it has been suggested that the semiquinone of these antitumor compounds are sufficiently stable to enter the nucleus, and either to intercalate into or react with DNA, or other macromolecules as a consequence of the affinity characteristics of these compounds.

The second mechanism of biochemical activation, for which considerable support has been established, focuses instead on the quinone-catalyzed superoxide or superoxide-derived species. Our work emphasizes that these two mechanisms can not be operating simultaneously, because superoxide generation necessarily destroys the semiquinone. Poorly perfused hypoxic tumors are possibly an important exception to this argument, because such tissues will have much lower levels of intracellular oxygen. This part of the project is concluded and has resulted in the publication below.

The anthracycline semiquinone spectra begin to change shape within minutes of their appearance. The symmetric signal first becomes asymmetric. Although the time course of further changes required 2 or 3 hr and is variable, the spectrum of the daunorubicin semiquinone acquired the features of a strongly-immobilized axially symmetric g tensor, characterized by $g_{\perp}$ and g_{11}, where g is equal to the trace of the tensor, $g=1/3(2g_{\perp}+g_{11})$. The final spectrum is characteristic of a semiquinone radical as evidenced by its g-value. In order to observe such a spectrum, the semiquinone must be rotating very slowly on the ESR time scale. Perhaps anthracycline polymerization products make the major contribution to the final immobilized spectrum.

This melanin like ESR signal is of considerable interest. We are attempting to obtain this ESR signal in the absence of microsomes by either photochemical or chemical reduction in order to ascertain the importance of lipid and/or protein binding to the appearance of this signal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Quinone compounds are very commonly used both in industry and medicine, as antibacterials and anticancer drugs. The redox metabolism of these compounds is thought to be

of importance in their mode of biological activities. This redox-mediated toxicity is not limited to bacterial or neoplastic cells but should effect all metabolically active cells.

PUBLICATIONS

Kalyanraman, B., Perez-Reyes, E., and Mason, R.P.: Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anti-cancer drugs. Biochem. Biophys. Acta. 630: 119-130, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50050-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

ESR Evidence for a Free Radical in the Cis-Trans Isomerization of Furylfuramide

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

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
Others:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

It has been proposed that the enzymatic cis-trans isomerization of furylfuramide is the result of anion free radical formation by nitroreductases. Electron spin resonance measurements of the furylfuramide anion free radical have provided direct spectral evidence for this intermediate, and clarified the disputed relationship between the isomerization and the nitro reduction of furylfuramide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectra of anaerobic microsomal incubations at 25° were obtained with a Varian century series E-109 spectrometer equipped with a TM_{110} cavity. Visible spectra of *cis*- and *trans*-furylfuramide (AF-2) were obtained with a DW-2A Aminco-Chance spectrophotometer at 37°.

MAJOR FINDINGS AND PROPOSED COURSE: Tatsumi *et al.* have proposed that enzymatic *cis-trans* isomerization of furylfuramide is a direct consequence of enzymatic nitro reduction. The nitroreductases, which are inhibited by oxygen, transfer a single electron to nitro substrates to give their respective anion free radicals. The carbon-carbon double bond linking the two furan rings of AF-2 would be weakened by anion radical formation, because the additional electron is in an antibonding molecular orbital. Upon formation, the *cis*-AF-2 anion free radical was proposed to isomerize rapidly to the *trans*-AF-2 anion, which could then be oxidized to form *trans*-AF-2.

Recently, Tomoeda and Kitamura studied the isomerization and nitro reduction of *cis*-AF-2 by subcellular fractions of *E. coli*. Their observations suggested that the nitro reducing and isomerizing activities of *E. coli* were due to totally different enzymes, in apparent contradiction of the isomerization mechanism of Tatsumi *et al.*

The ESR spectrum of an anaerobic microsomal incubation containing furylfuramide and an NADPH-generating system provides direct evidence of free radical formation. In the presence of air, nitroaromatic anion free radicals undergo rapid air oxidation to form superoxide anion, and the nitroaromatic anion free radicals not detected. In addition, identical spectra were obtained with either *cis*- or *trans*-AF-2. These ESR spectra did not vary with time even though visible spectroscopy shows a rapid and nearly complete conversion of *cis*-AF-2 to *trans*-AF-2.

Although spectral evidence alone indicates that both the major and the minor components of the spectrum represent AF-2 anion free radicals, the assignment of the spectrum to *cis-trans* anion free radicals must be made on other grounds. INDO (intermediate neglect of differential overlap) molecular orbital calculations on *cis*- and *trans*-AF-2 anions suggest that the *cis* anion radical will have the larger nitrogen hyperfine splitting constant. Furthermore, the greater steric hindrance expected for the *cis*-conformer should result in a predominance of the *trans*-conformer. In the parent compounds, the greater thermodynamic stability of *trans*-AF-2 (87-91%) vs that of *trans*-AF-2 to *cis*-AF-2 (7-11%) observed in xanthine oxidase incubations under optimum conditions. Clearly, since xanthine oxidase is only a catalyst, and cannot change the thermodynamic equilibrium of *cis*- *trans*-AF-2, the equilibrium ratio of *cis*- to *trans*-AF-2 and the two free radicals is the strongest chemical evidence for our assignment of the two species as the respective anion radicals. The observation of distinct conformational isomers with ESR only requires that the equilibrium mixture be slowly interconverting on the ESR time scale, which is very rapid. More precisely, the lifetime of the conformers must be much longer than $1/\gamma_e (a^N_{cis} - a^N_{trans}) = 28$ nanoseconds.

TThe reaction can still be fast in terms of chemical reaction, and in fact, competes favorably with the autoxidation of the AF-2 anion free radicals.

The results of Tomoeda and Kitamura and the isomerization mechanism of Tatsumi *et al.* are not contradictory if the oxygen-insensitive *E. coli* reducing activity does not form the anion radical intermediate and the oxygen-sensitive *E. coli* reducing activity does form the anion radical, as has been recently demonstrated. In the present study, we provide electron spin resonance evidence for *cis*- and *trans*-AF-2 anion radical intermediate formation during the microsomal *cis-trans* isomerization of AF-2.

Higher resolution ESR investigations in D_2O buffer, in conjunction with INDO calculations, support this concept by unambiguously demonstrating the enzymatic generation of *cis* and *trans* radical anions of 3-(5-nitro-2-furyl)-2(2-furyl) acrylamide. The INDO calculations further indicate that the rotational barrier between the *cis* and *trans* anion radicals of this compound is only 5-10 kcal/mole, whereas a 70 kcal/mole barrier exists for the parent geometric isomers. Hyperfine splitting constants for the *cis-trans* conformers have been assigned on the basis of INDO calculations. Surprisingly, only the nitrogen hyperfine splitting of the nitro group is distinguishably different in the two conformers, a result which is not inconsistent with the INDO calculations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Furylfuramide has been shown to be mutagenic, carcinogenic and cytotoxic. Enzymatic nitro reduction of furylfuramide is thought to form the reactive metabolites which are primarily responsible for these effects. The synthetic form of this former food additive is the *cis* isomer. Typical mammalian nitroreductases, such as xanthine oxidase or rat liver microsomal nitroreductase, isomerize *cis*-furylfuramide to *trans*-furylfuramide before they initiate reductive "activation". Many investigators have observed this isomerization in the course of investigations of the reductive activation of *cis*-AF-2 by mammalian and bacterial systems.

PUBLICATIONS

Kalyanaraman, B., Mason, R.P., Rowlett, R., and Kispert, L.D.: An electron spin resonance investigation and molecular orbital calculation of the anion radical intermediate in the enzymatic cis-trans isomerization of furylfuramide, a nitrofuran derivative of ethylene. Biochim. Biophys. Acta (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50051-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Free Radical Metabolism of Polycyano Compounds

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

PI: Ronald P. Mason Research Chemist LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Polycyano compounds have been proposed for use as superconductors in high voltage transmission lines. These compounds are known to be strong electron acceptors which also form charge-transfer complexes. It is the objective of this study to examine the biological properties of polycyano compounds and to determine their metabolic fate. Preliminary experiments have shown that in microsomal incubations the electron transfer between tetracyanobenzene and some unknown biological donor is complete and the radical anion of this compound is formed. The electron transfer to form the tetracyanobenzene anion radical is dependent upon the presence of NADPH. In future studies, the source of the electron will be sought and the microsomal metabolism of other polycyano compounds will be examined.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy was employed to detect steady-state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: Tetracyanobenzene was metabolized to an anion free radical by rat liver microsomes. The ESR spectrum of this free radical metabolite was dependent upon NADPH. We now intend to determine what other metabolites are formed by this reduction path, because microsomal incubations acquire a pink color due to a metabolite, which is not a free radical. Cyanide is also expected to be a reduction metabolite, because it could form by reductive cleavage of the phenyl-nitrile bond. This project is not progressing due to our inability to obtain pure tetracyano benzene.

Next, we will investigate the source of the single electron which was transferred to tetracyanobenzene. The main possibilities are cytochrome P450 and b_5 and their respective flavin-containing reductases, NADPH-cytochrome P-450 and NADPH-cytochrome b_5 reductase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tetracyanocompounds are used in organic superconductors, which are thought to have great potential in the transmission of high voltages without power loss. Although these compounds are reported to have fungicidal and bactericidal properties there is little or no information on their other biological effects and nothing is known about their toxicity and metabolic fate.

PUBLICATIONS

Mason, R.P.: Free radical metabolites of toxic chemicals. Pryor (Ed.): Free Radicals in Biology, Vol. V, Academic Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50052-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Binding of Chemically Activated Semiquinone Free Radicals from Anticancer Agents to DNA

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

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Chemical reduction of the highly active quinone-containing antitumor drugs, adriamycin and daunorubicin formed the same partially reduced free radical previously reported by microsomal activation. *In vitro* incubation of the chemically activated free radical intermediates with DNA resulted in covalent binding of these drugs to DNA. The adriamycin semiquinone radical has a greater affinity for DNA and covalent complexes containing up to one adriamycin per 15 nucleotides were obtained. The daunorubicin semiquinone radical, on the other hand, showed a lesser binding affinity and gave rise to complexes in which one drug molecule was covalently bound per 140 nucleotides. Studies with synthetic polynucleotides suggest that these drugs have a high preference for poly (dG) and poly (dC). Microsomal activated drugs also bind covalently to DNA with identical binding affinities. Adriamycin, when injected in rats, also bind covalently to rat liver proteins, RNA and DNA. Microsomal activation of these drugs produced both C_7-free radical and C_7-quinone methide which act as alkylating agents.

PROJECT DESCRIPTION

METHODS EMPLOYED: We have previously shown that chemical reduction ($NaBH_4$) of adriamycin and daunorubicin generated same free radical intermediates as previously reported by microsomal activation. Incubation of the free radical intermediates with DNA, in vitro, resulted in covalent binding of these drugs to DNA. Adriamycin had a greater binding affinity for DNA than daunorubicin which correlates with their ability to indule sister chromatid exchanges. We have also shown that the microsomal-NADPH activated drugs covalently bind to nucleic acids and that this binding decreases with increasing incubation time prior to the addition of DNA.

MAJOR FINDINGS AND PROPOSED COURSE: Recently we have examined the binding of ^{14}C-adriamycin to cellular macromolecules in vivo. Intraperitoneal injection of ^{14}C-adriamycin to rats results in covalent binding of the durgs to protein, RNA and DNA. Our results show that more adriamycin is bound to protein and to RNA than DNA at each point. Furthermore, the binding decreases rapidly with time indicating that an enzymatic repair process may be operating. Although, the species that bind(s) to the cellular macromolecules is not known, Moore and we have suggested that C_7-quinone methide and/or C_7-free radical metabolite of adriamycin may act as active alkylating species. Recently, we have examined the formation and binding of these one electron (C_7-free radical) and two electron (C_7-quinone methide) reduction products of adriamycin and daunorubicin. Our findings show that microsomal-NADPH reduction of these drugs produces both one and two electron reduction product. Furthermore, the C_7-quinone methide also binds to DNA. Future plans include characterization of the adduct by enzymatic degradation and a study of the binding of C_7-free radical and C_7-quinone methide to soluble SH-compounds (GSH, cystein).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the mechanism of action of antitumor drugs and chemical carcinogens stems from their ability to interact with nucleic acids, it is of great significance to understand such binding at a molecular level. It is hoped that by defining these interactions, a better understanding of the tumorigenicity will result.

PUBLICATIONS

Sinha, B.K. and Sik, R.H.: Binding of ^{14}C-adriamycin to cellular macromolecules, in vivo. Biochem. Pharmacol. 29: 1867-1868, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50053-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Binding of Antitumor Drugs to Membranes

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

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB NIEHS
OTHERS:	Mary J. Ortner	Senior Staff Fellow	LEB NIEHS
	Colin F. Chignell	Chief	LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The cytotoxic and mutagenic properties of antitumor drugs such as adriamycin, acridines, diacridine, actinomycin D and Pt compounds are related to their interaction with nucleic acids and inhibition of protein systhesis. We have examined their interaction with human erythrocyte ghost membranes and murine mastocytoma cells using spin labeling techniques. These drugs induce changes in electron spin resonance of the spin labeled ghost membranes and in the mastocytoma cells. These findings suggest that these drugs induce changes in protein conformation of the membranes. Using cytofluorescence technique, we have shown that the binding of adriamycin leads to the protein aggregation in Hut-II cells. Thus, membrane binding properties of these drug may be important in their mechanism of action.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHOD EMPLOYED: In previous studies we have shown that a number of antitumor drugs (which bind to nucleic acids and are also carcinogenic), interact with membranes. We also found, using spin labeling techniques, that these drugs induce changes in red cell ghost membranes and mastocytoma P815 cells suggesting alteration of their protein conformation. These findings may be related to the cytotoxicity of these agents since alterations in protein conformations may be expected to affect many physiological functions (ions transport, glucose transport, etc) of the cells.

MAJOR FINDINGS AND PROPOSED COURSE: We have now examined the binding of anthracycline antibiotics, adriamycin and daunomycin, to cell membranes using fluorescence microscopy. Incubation of the drugs (10^{-6}M, 10^{-5}M) with human ghost membranes, followed by removal of the unbound drugs by washing, resulted in a bright yellow membrane fluorescence indicating that the drugs were bound. In live cells (mastocytoma 815 and Hut II), incubation of either adriamycin or daunomycin for 5 min at 37°C resulted in a faint yellow fluorescence apparently associated with the nuclear membranes. Furthermore, only a bright yellow fluorescence was detected after 4 hrs of incubation. These observations do not support earlier observations of Krishan, et al. and Bachur, et al. who have shown that these drugs bind to nucleic acids and appear as red-orange fluorescence. Furthermore, Krishan, et al. had shown that this adriamycin-specific red-orange fluorescence appears readily in dead cells. Our observations then suggest that either the binding of adriamycin does not lead to cell death or the binding does not produced red-orange fluorescence. Counterstaining with trypan blue, which is excluded by the live cells, however, resulted in both yellow and red fluoresence. Furthermore, the yellow fluorescence was exclusively associated with the live cells while the red fluorescence was observed in the dead cells. In addition, a distinct patching of the yellow fluorescence was detectable in these cells suggesting that the binding of adriamycin leads to protein aggregation. The observation that the red fluorescence is always associated with cells that have taken up trypan blue suggests that this red fluorescence originates from trypan blue which is excited via non-radiative energy transfer from adriamycin.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the carcinogenic and mutagenic properties of antitumor drugs are most likely associated with DNA binding, it is of great significance to understand the mechanism of actions of these drugs at a molecular level. It is hoped that such studies may lead to better understanding of the chemical properties that are responsible for the carcinogenic and antitumor properties of these agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50054-03 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Free Radical Formed Microsomal Incubations Containing CCl_4 and NADPH

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

PI:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
OTHERS:	R.P. Mason	Research Chemist	LEB	NIEHS
	C.F. Chignell	Chief	LEB	NIEHS
	C.R. Wolf	Visiting Associate	LP	NIEHS
	R.M. Philpot	Research Chemist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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 (200 words or less - underline keywords)

The hepatotoxicity of carbon tetrachloride is usually thought to be due to the enzymatic formation of the trichloromethyl radical. A variety of indirect, but not conclusive, evidence for the formation of $^{\cdot}CCl_3$ exists: hydrogen abstraction by $^{\cdot}CCl_3$ to form $CHCl_3$ and dimerization of $^{\cdot}CCl_3$ to form C_2Cl_6. Hydrogen abstraction of a methylene hydrogen from polyunsaturated fatty acids by the trichloromethyl radical would be followed by oxygen addition and would result in lipid peroxidation. Carbon tetrachloride-induced lipid peroxidation has been extensively studied both in vitro and in vivo. Attempts to use electron spin resonance (ESR) spectroscopy to demonstrate directly the presence of the trichloromethyl radical in hepatic microsomal incubations or liver slices have been unsuccessful. Recently a free radical has been detected in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-t-butyl nitrone (PBN). This free radical adduct was identified as the $^{\cdot}CCl_3$ adduct of PBN. Our studies have shown, however, that a lipid dienyl radical, similar to that formed by the action of soybean lipoxygenase on linoleic acid, is the species being trapped.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy, in conjunction with spin-trapping agents, has been used to detect steady state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: With the aid of the spin-trapping technique, Poyer, et al. have detected a free radical in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-t-butyl nitrone (PBN). This free radical was identified as the trichloromethyl adduct of PBN based on the similarity of its ESR spectrum to that of the free radical formed by UV photolysis of a CCl_4 solution of PBN. Ingall et al. using the spin trap 2-methyl-2-nitroso-propane (MNP), have also trapped a free radical in microsomal incubations containing CCL_4 and NADPH, but in this case, the spectrum was not the same as that generated by the X-ray irradiation of a CCl_4 solution of MNP. The latter spectrum was claimed to be that of the MNP-trichloromethyl spin adduct based on an isotopic effect seen with ^{13}C-carbon tetrachloride. However, it should be noted that this spectrum of the MNP-trichloromethyl radical adduct is clearly different from earlier spectra in that hyperfine structure due to chlorine was not observed. Based on the differences between the microsomal and X-ray irradiation spectra, Ingall et al. concluded that the free radical trapped in the microsomal experiments was probably either CCl_3-$O_2^{\cdot}$ or a secondary lipid peroxyl radical rather than the $^{\cdot}CCl_3$ radical. Our spin-trapping investigations with PBN and MNP have demonstrated that these interpretations are in error, and that in both cases a lipid dienyl free radical, similar to that formed by the action of soybean lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) on linoleic acid, is probably the species that is trapped.

Further investigations with MNP and PBN are in progress. Preliminary results indicate the MNP-carbon centered lipid adduct may be a result of a non-enzymatic nonfree radical reaction of MNP with the microsomal lipids.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: CCl_4 toxicity results from metabolic activation of this agent by the liver, which is the main site of CCl_4-induced pathological changes. This activation is thought to require the homolytic cleavage of one of the chlorine-carbon bonds of CCl_4, to form the trichloromethyl free radical. From this single unproven event the entire spectrum of pathological consequences of CCl_4 poisoning is thought to follow. The central importance of this proposed free radical metabolite to the hepatotoxicity of CCl_4 makes a demonstration of its existence in a biological system of considerable importance.

PUBLICATIONS

Wolf, C.R. Harrelson, W.G., Jr., Nastainczyk, W.M., Kalyanaraman, B. and Mason, R.P.: Metabolism of carbon tetrachloride in hepatic microsomes and reconstituted monoxygenase systems and its relationship to lipid peroxidation. Mol. Pharm. 18: 553-558, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50055-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Binding of Reductively Activated Streptonigrin to Nucleic Acids in the Presence of Metals

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

PI: Birandra K. Sinha Senior Staff Fellow LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The binding of streptonigrin (SN) to nucleic acids was studied in the presence of reducing agents and metals. Incubation of chemically reduced SN with DNA in vitro resulted in irreversible binding and complexes containing 1 mole of SN per 250 nucleotides were obtained. The presence of Zn^{2+} increased this binding considerably to give complexes containing 1 mole of SN per 80 nucleotides. On the other hand, Mg^{2+} decreased this binding. More drug was bound to denatured DNA than to native DNA. Maximum binding was obtained when SN was reduced in the presence of DNA. Increased binding was also obtained when the fully reduced SN was incubated with DNA. Furthermore, enzymatically activated SN binds to DNA to a greater degree than SN activated by chemical systems. Studies with synthetic polynucleotides in the presence of Zn^{2+} suggested that while SN has a high affinity for guanine, cytosine and adenine also serve as excellent substrates. These studies indicate that the active intermediate that binds to nucleic acids is unstable and may be derived from the fully reduced drug. These in vitro studies further suggest that Zn^{2+} plays an important role in the binding of SN to DNA and may have implications for the biological actions of SN if similar reactions occurred in vivo.

PROJECT DESCRIPTION

MAJOR FINDING AND PROPOSED COURSE: Our earlier studies have shown that SN binds irreversibly to DNA in the presence of a reducing agent (chemical or enzymatic). This binding, however, is greatly enhanced by the presence of ZN^{2+}. Although SN, in the presence of $NaBH_4$ and ZN^{2+}, show a high affinity for guanine bases, cytosine and adenine also are excellent substrate for this binding. Our studies also show that more SN is bound to the denatured DNA than the native DNA.

The species that bind to DNA is not known, however studies indicate that it is unstable and may be derived from the fully reduced drug.

This project is essentially complete and no future work is planned at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In conclusion, I have shown that in the presence of a reducing agent (either chemical or enzymatic) streptonigrin binds irreversibly to DNA. The presence of Zn^{2+} and reducing agents greatly enhance this binding. The activated SN in the presence of Zn^{2+} shows high affinity for guanine, however, cytosine and adenine also serve as excellent substrates. Binding of SN to DNA, under reducing conditions *in vivo* and in the presence of divalent ions (ZN^{2+}, CU^{2+}, etc), may then explain the strand breaks induced by this drug. In addition, such strong binding of SN to macromolecules may also have implications in its mutagenecity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50056-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of hypothermia on ion movement in guinea pig cochlea

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

PI:	Teruzo Konishi	LEB	Medical Officer (Research)
	Alec N. Salt	LEB	Visiting Fellow
OTHER:	Philip E. Hamrick	SFTY	Radiation Safety Officer

COOPERATING UNITS (if any)

Radiation Safety

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

It has been documented that the cochlear potentials are maintained by metabolic energy. However, it is not known whether a decrease of metabolic rate by hypothermia can alter ion movement or membrane permeability of the cochlea. The present study is designed to determine dependence of cochlear membrane permeability on temperature.

PROJECT DESCRIPTION

METHODS EMPLOYED: The cochlear potentials were recorded from the basal turn of the cochlea of guinea pigs anesthetized with sodium pentobarbital. The rectal temperature and blood pressure in the common carotid artery were monitored. The rectal temperature was reduced from 39° ± 0.5°C to 29° ± 0.5°C with a cooling pad. The perfusion of the perilymphatic space with solutions containing ^{43}K and ^{22}Na and collection of the cochlear fluids were carried out during steady state hypothermia. Total concentrations of K^+ and Na^+ in the cochlear fluids were determined by a helium glow photometer and radioactivities of ^{43}K and ^{22}Na were determined by gamma spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potenitals. The endocochlear potential (EP) measured at 39°C was 86.0 ± 3.5 mV. When the rectal temperature decreased to 29°C the EP was 78.2 ± 3.5 mV. The EP recorded 2 hours after the rectal temperature was kept at 29.0° ± 0.5°C was 73.2 ± 3.9 mV. During the course of hypothermia the magnitude of cochlear microphonics (CM) gradually decreased. Usually a large increase of the negative summating potential accompanied the decrease in CM during the period of hypothermia. The action potential (AP) in response to test stimuli of low intensity were markedly suppressed but the AP remained little changed or became supernormal with high intensity stimuli.

2. Electrolyte concentrations in the cochlear fluids. Hypothermia did not result in marked changes in K^+ concentrations in the endloymph and perilymph of nonperfused cochlea. The Na^+ concentrations in both endolymph and perilymph were slightly decreased in hypothermic guinea pigs.

3. Membrane permeability of the endolymph-perilymph barrier determined by rate constant for K. When the perilymphatic space was perfused with radioactive artificial perilymph, the ^{43}K concentrations in the endolymph (normalized by ^{43}K concentrations in the perilymph) increased exponentially. The rate constant for K^+ was 0.0069 min^{-1} in hypothermic guinea pigs which was significantly lower than the value obtained in normal guinea pigs (0.013 min^{-1}).

The K^+ conductance of the endolymph-perilymph barrier can be computed by using the following equation

$$G_K = \frac{\lambda_K F^2 V_{end} [K^+_{end}]}{\Delta\tilde{\mu}_K}$$

where V_{end} is volume of the endolymph (2.1) and $\Delta\tilde{\mu}_K$ is the electrochemical potential difference between endolymph and perilymph. The computed G_K was 20.65 x 10^{-6} mho in hypethermic guinea pigs which was considerably lower than 35.16 x 10^{-6} mho in normal guinea pigs. The permeability constant for K^+ (P_K) was 133.54 x 10^{-9} cm^3 sec^{-1} in hypothermic guinea pigs. This value was substantially lower than the normal value (242.9 x 10^{-9} cm^3 sec^{-1}).

4. Membrane permeability of the endolymph-perilymph barrier determined from K^+ passive diffusion during anoxic period. Permanent anoxia was induced 30 min after the rectal temperature fell to 29.0°C. When anoxia continued for longer than 5 min the decrease of the endolymph K^+ concentration measured with K^+ selective liquid membrane electrodes was solely attributable to a passive K^+

diffusion. The K^+ conductance was calculated from the rate of change of the endolymph K^+ concentration relative to the K^+ electrochemical potential difference. The mean G_K averaged from 10 to 30 min after onset of anoxia was $(21.53 \pm 5.54) \times 10^{-6}$mho in hypothermic guinea pigs, whereas the mean G_K averaged during the same period was $(34.85 \pm 5.60) \times 10^{-6}$mho in normal guinea pigs.

A manuscript has been accepted for publication and this project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The present data suggest that hypothermia results in changes in both active and passive ion transport mechanisms in the cochlea. It is likely that a decrease of metabolic energy caused by ototoxic insults may interfere not only with active ion transport, but also the membrane permeability of the cochlear partition.

PUBLICATIONS

Konishi, T., Salt, A.N. and Hamrick, P.E.: Effects of hypothermia on ion movement in guinea pig cochlea. Hearing Res. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50057-02 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Microwave Radiation on Reproductive Cells

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

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Cindy H. Hall	Graduate Student	N.C. State University	
	J. Paul Thaxton	Poultry Science Dept.	N.C. State University	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle, N.C.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

In this study, the effect of non-ionizing radiation on the integrity of mature spermatocytes was examined. Semen was obtained from 10-month-old turkeys and dilluted to a concentration of 3.5 x 10^8 sperm/ml using Beltsville Poultry Semen Extender. The sperm were exposed for 30 minutes to 2.45 GHz microwave radiation at specific absorption rates (SAR's) of 0, (sham) 10 or 50 mW/g. The waveguide exposure system maintained the control (non-irradiated) and irradiated samples at 40 ± 0.15°C throughout the experiment. Microwave radiation did not alter membrane permeability to a vital stain or the intracellular enzymes LDH and GOT. The in vivo performance of the sperm following in vitro irradiation was also examined. The fertility of virgin turkey hens given a single insemination of microwave exposed sperm was similar for the 10 mw/g and nonexposed sperm. Hatachability and embryonic mortality were not different among the treatment groups. A number of hematological parameters in the progeny of the turkey hens are now being examined.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: a. Cell Preparation: Semen was obtained from 7-month-old Nicholas large white turkeys. Biological variation was minimized by pooling semen from 30 turkeys. For each experiment, 2 ml of semen were collected and diluted 2:1 with Beltsville Poultry Semen Extender, BPSE (USDA, Beltsville, MD). The semen was washed twice to remove the seminal plasma. The sperm were then resuspended in BPSE to a concentration of 5.0×10^8 sperm/ml. The sperm were irradiated in an S-band waveguide chamber at a frequency of 2450 MHz. Each tube contained 4.2 ml of the sperm suspension. The tubes were siliconized to minimize cellular adhesion and were gently stirred throughout the exposure duration to allow uniform microwave absorption and temperature distribution.

b. Cellular Integrity: Several parameters were selected as indices of altered membrane function: permeability to a vital stain (viability) release of lactate dehydrogenase (LDH) and release of glutamic oxalic transaminase (GOT).

c. In vivo performance of sperm following in vitro irradiation: Virgin turkey hens were given a single insemination of sperm which were either sham-exposed, or exposed to 10 mw/g or 50 mw/g. Egg fertility was determined during the nine weeks following insemination.

d. Evaluation of progeny from turkey hens receiving microwave irradiated sperm. Hematological evaluation of the progeny from eggs laid during the second and fourth weeks after insemination. The turkey polts were evaluated a 2, 4 and 5 weeks of age.

MAJOR FINDINGS AND PROPOSED COURSE: a. Cellular Integrity: Initially, the viability was 96% and was not affected by microwave radiation at any of the exposure levels examined. The release of the soluble enzymes, lactic acid dehydrogenase, and glutamic oxalic transaminase, by sperm into the suspending media was not altered.

b. In vivo performance of sperm following in vitro irradiation: Fertility declined comparably in all groups until the fourth week after insemination. During the fourth week, fertility increased in the hens which received 50-EXP sperm and elevated fertility was maintained in this group for the duration of the experiment. Hatchability and embryonic mortality were not different.

c. Evaluation of progeny from turkey hens receiving microwave irradiated sperm: No data available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment are of interest to the National Institute of Environmental Health Sciences. Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control the temperature of the specimen carefully and be able to reproduce the exposure conditions. We have developed the capability to do this for in vitro exposures at NIEHS and this may provide a system for differentiating specific microwave effects from thermal responses. In addition, by examining the response of cells and cellular components to microwave radiation,

it should be easier to identify the mechanisms of action of microwave radiation with biological specimens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50058-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Microwave Effects on Fetal Development in Mice

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

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
	Peter Nawrot	Visiting Associate	LEB	NIEHS
OTHER:	Minuro Inouye	Visiting Fellow	LEB	NIEHS
	R.E. Staples	Research Scientist	Dupont Corp., Wilmington, Del.	

COOPERATING UNITS (if any)

Research Triangle Institute

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Pregnant mice (CD-1 strain) were exposed to 2.45 GHz microwave radiation at a power density level of 30 mW/cm². At exposure to 30 mW/cm² (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/cm² 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/cm².

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this research was to determine the maternal and embryotoxic effects of microwaves. In order to determine whether or not the effects were only thermal or a combination of thermal and specific microwave interactions, groups of animals were placed in elevated temperature environments in order to simulate the thermal stress of the microwave exposure. The mice were exposed from above in styrofoam cages (one animal per cage) separated at least 2 body lengths with the long axis of the cages parallel to the electric field.

MAJOR FINDINGS AND PROPOSED COURSE: We repeated the experiment which exposed pregnant CD-1 mice to 30 mW/cm^2 to 2.45 - GHz microwave radiation. Our investigation showed that exposure to 30 mW/cm^2 during days 1-6 produced a signficant decrease in implantation sites per litter and average fetal weight. In our 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.

The research project will be completed during this fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: Sufficient information is not available at the present time to establish scientifically based safety standards for microwave radiation exposure. The determination of the intensity of microwave radiation which produces teratogenic effects is important to the evaluation of the hazardous effects of microwaves. This research is part of the mission of the Institute to conduct research on the health effects of physical factors in the environment.

PUBLICATIONS

Nawrot, P.S., McRee, D.I. and Staples, R.E.: Effects of 2.45 GHz microwave radiation on embryofetal development in mice. Teratology (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50059-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protoporphyrin Phototoxicity in Rat Mast Cells and Human Erythrocytes

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

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHER:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Protoporphyrin-mediated phototoxicity has been studied in human erythrocytes and rat mast cells. Circular dichroism studies of erythrocyte ghosts have shown that phototoxic lysis is accompanied by membrane protein denaturation with loss of alpha helical structure. Rat mast cells showed a dual phototoxic response which depended on the light intensity. Low intensity light caused a stabilization of the cell membrane resulting in a loss of histamine secretory ability; whereas high intensity light caused phototoxic lysis. Sodium dodecyl sulfate disc gel electrophoresis indicated that the mast cell membrane proteins are covalently crosslinked by the phototoxic reaction in a manner similar to that seen in erythrocyte ghosts. Biophysical studies are currently underway to establish the molecular mechanisms of these phenomonea.

PROJECT DESCRIPTION

OBJECTIVES: Severe phototoxic reactions may be clinically manifested in patients with erythropoietic porphoryia, a metabolic disease which results in a high buildup of porphyrins in the blood. Porphyrin-mediated phototoxicity is related to erythrocyte hemolysis, and in spite of extensive biochemical studies, the molecular mechanism is not completely understood. We have studied this phototoxic reaction in erythrocyte ghosts using circular dichroism (CD), a technique which is sensitive to membrane protein conformation. In addition, we have studied the reaction in a eukaryotic cell which can be stimulated in vitro to perform a biological response. The purpose of this approach is to study more closely the intermediate oxidative reactions which precede erythrocyte lysis, and to determine the effects of these reactions on erythrocytes and functioning eukaryotic cells.

METHODS EMPLOYED: Purified rat peritoneal mast cells and human erythrocyte ghosts were obtained using well established methods. Cells and ghosts were exposed to light in the presence of protoporphyrin using either a 100W incandescent light bulb or a 100W mercury vapor lamp. The light intensity was varied by changing the distance from the light source. Circular dichroism measurements were performed on erythrocyte ghosts exposed to light and protoporphyrin using a Jasco automatic recording polarimeter. Simultaneous measurements of membrane protein optical density at 280nm indicated that changes in the circular dichroism spectra were not due to scattering artifacts.

MAJOR FINDINGS: Human erythrocytes and ghosts. Human erythrocytes exposed to strong light in the presence of protoporphyrin underwent lysis on a time-dependent basis. An equivalent number of ghosts exposed under identical conditions showed changes in CD spectra of the membrane proteins which were consistent with loss in α-helical structure due to protein denaturation. Appropriate controls showed that the effect was dependent both on protoporphyrin and light. Changes in the CD spectra began after 2-4 min when the ghosts (20 μg Protein/ml) were exposed to 2mM protoporphyrin and placed 20 cm from the mercury vapor light source. In the intact erythrocytes, phototoxic lysis began between 6-8 min after the begining of illumination and progressed with time in a sigmoidal fashion until almost 100% lysis occurred. The data therefore indicate that changes in the secondary protein structure of erythrocyte ghost proteins can be correlated with protoporphyrin induced photolysis.

Although D_2O decreased the rate of lysis in erythrocyte ghosts, there were no effects on the rate of decrease in membrane protein secondary structure as determined by circular dichroism. This suggests that either singlet oxygen does not play a significant role in protoporphyrin phototoxicity or that in this system, singlet oxygen does not decay via collisions with the aqueous environemnt.

Rat Peritoneal Mast Cells. Rat mast cells undergo lysis when exposed to protoporphyrin and high intensity light. This reaction is manifested by the cytotoxic release of histamine due to conditions which may be similar to those seen in erythrocyte ghosts. This study has shown, however, that mast cell membranes do not lyse when exposed to protoporphyrin and low intensity light, but rather are stabilized in a way which makes them resistant to histamine liberators. The

development of this inhibition is both dose and time dependent (100 μg/ml protoporphyrin for 30 min gives total inhibition) and does not occur in the dark or under ordinary room light. Purified mast cells were exposed to conditions which produced inhibition, and the proteins separated using SDS-disc gel electrophoresis. Preliminary results indicated that some of the proteins were unable to enter the gel, presumably due to crosslinking and aggregation. This would indicate that the stabilization phase of protoporphyrin phototoxicity in mast cells may involve a protein crosslinking similar to that which accompanies erythrocyte lysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Severe phototoxic urticaria can occur as a result of clinical disorders such as erythropoietic porphyria, from direct phototoxic reactions to drugs and chemicals or from photoallergic reactions to haptens. A biophysical study of phototoxicity in erythrocyte membranes has not been previously undertaken. Protoporphyrin-induced phototoxicity is therefore being studied as a model system in order to understand more clearly the role of active oxygen in the phototoxic response. The use of these techniques will contribute to our knowledge of both membrane physiology and phototoxicity. The mast cell provides an excellent model for both in vitro and in vivo studies of phototoxicity in a eukaryotic cell. Furthermore, mast cells secrete several mediators of inflammation and since they occur abundantly in the skin, they may play a direct role in the phototoxic response. A thorough study is therefore needed to understand the molecular mechanism of this response in the mast cell. These studies may lead to a method of control over the symptoms of phototoxic urticaria.

PROPOSED COURSE: We are currently developing techniques to study the effects of several agents on the early development of membrane protein denaturation and the involvement of specific ghost proteins. Since protoporphyrin phototoxicity is probably mediated via reduced forms of active oxygen, we have studied the early stages of phototoxicity in the presence of agents which are known to affect these forms (D_2O superoxide dismutase, mannitol, catalase, etc.) The use of circular dichrosim makes it possible to study the time course of membrane protein denaturation directly and also to study this reaction in the early, pre-lytic stages of phototoxic damage.

The effects of protoporphyrin phototoxicity on membrane intercalated spin labels will be studied using ESR. Lipid spin labels can be intercalated into erythrocyte ghosts to probe the interface regions and the hydrophobic interior. In addition, the membrane proteins may be covalently spin labeled and the phototoxic reaction studied at this level. Because the spin probes are degraded by the strong oxidants produced during the phototoxic response, it may be possible to determine the area in the membrane where the greatest number of oxidative reactions are occurring, and whether this area changes during the course of the reaction.

Protoporphyrin mediated phototoxicity has not as yet been studied directly in erythrocyte ghosts. Using the biophysical techniques outlined above, we hope to learn more about this phototoxic response and the secondary reactions, it precipitates in the erythrocyte membrane.

We plan to continue these studies of phototoxicity in mast cells by studying the

reaction in the presence of inhibitors and quenchers of reduced forms of active oxygen. Although histamine release is affected by some of these agents, the effects on protein crosslinking may still be determined. In this way, we may learn whether the phototoxic effect of protoporphyrin is mediated directly or via active oxygen in the mast cell. The membrane stabilizing effects will also be studied in erythrocytes exposed under similar light conditions. This will determine whether erythrocytes also undergo a dual reaction to protoporphyrin and light.

In vivo studies will also be done which will determine the effects of porphyrins on mast cells. Protoporphyrin will be administered to rats in a manner known to cause phototoxic reactions. The mast cells will be removed from these animals and examined for morphological differences, viability and responsivity to histamine liberators. This project may provide information regarding the role of the mast cell in the phototoxic response.

PUBLICATIONS

Ortner, M.J., Abhold, R.H. and Chignell, C.F.: The effect of protoporphyrin on histamine secretion by rat peritoneal mast cells: a dual phototoxic reaction. Photochem. Photobiol. 33: 355, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50060-02 LEB

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Microwave Interactions with Cells and Cellular Components

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

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Cynthia Hall	Graduate Student	N.C. State University	
	Melvyn Lieberman	Consultant	Physiology, Duke University	
	J. Paul Thaxton	Consultant	N.C. State University	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIEH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and subcellular level, to observe any effects of this interaction, and to relate the amount of microwave energy absorbed to the effect. The biological specimens include macrophages, cardiac cells, lysosomes, and cellular enzymes. For these experiments, a waveguide exposure apparatus has been used which can maintain the specimens at physiologic temperatures (37°C ± 0.25°C) at specific absorption rates up to 100 mW/g. No effects were noted on in vitro activity of (creatine phosphokinase and acetylcholinesterase) at SAR's up to 50 mW/g. Embryonic cardiac cells obtained from 9-day old quail, exhibited an increase in cell membrane permeability to typan blue at SAR's of 10, 50 and 100 mW/g, following 90 minutes exposure. Cellular damage was noted at an SAR of 100 mW/g. Lysosome fragility was not influenced by microwaves at SAR's up to 100 mW/g and exposure durations of 90 min. Methods for examining the influence of microwave radiation on mitochondria and macrophages are being developed.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cells and cellular components were exposed to 2450 MHz microwave radiation using a waveguide exposure apparatus developed in this laboratory. Specimens were exposed to SAR's of 1, 10, 50 and 100 mW/g for up to 90 minutes. The samples exposed include mast cells (see Z01 ES 50039-02 LEB) cardiac cells, lysosomes, and enzymes. For each specimen certain parameters were monitored to determine the effects of microwaves on the biological specimen. In addition, when appropriate, election microscopy techniques were employed to determine microwave effects.

MAJOR FINDINGS AND PROPOSED COURSE: a. Cardiac cells. Isolated cardiac muscle cells were exposed to microwave radiation in a temperature controlled waveguide apparatus. Microwave radiation for 90 minutes at specific absorption rates (SAR) as low as 10 mW/g increases the permeability of cardiac cells to trypan blue. At 100 mW/g the inability of the cells to exclude trypan blue is concurrent with the release of lactic dehydrogenase into the suspending medium. However, when the SAR is decreased to 50 mW/g trypan blue uptake is increased without release of lactic dehydrogenase. Transmission electron micrographs of the exposed cells showed cellular damage only at the 100 mW/g exposure level. The effect on trypan blue permeability was unrelated to the macroscopic heating effect of microwave radiation on the cells, but may be due to some other specific action of microwave radiation on isolated cardiac cells. These studies have been extended to include other cell types such as macrophages, and spermatocytes (see Z01 ES 50057-02).

b. Lysosomes. At specific absorption rates of 10, 50 and 100 mW/g for 90 min at 37°C, no effects were noted on lysosomal fragility as determined by the release of the lysosomal enzymes, cathepsin D and β-glucuronidase. Furthermore, microwave exposure of the lysosomal suspension adjusted to pH 5.0, had no effect on the acid induced lysosomal enzyme release. The data from this study demonstrates that microwave radiation has no labilizing effect on lysosomal membranes although other microwave-membrane interactions not associated with enzyme release may occur.

These studies will be extended to include other aspects of lysosome physiology such as the response to drugs, and toxic compounds. In addition other organelles such as mitochondria will be studied.

c. Enzymes. The effect of 2.45 GHz microwave radiation on the *in vitro* activity of acetylcholinesterase and creatine phosphokinase was examined. The enzyme activities were determined during exposure to microwave radiation at specific absorption rates of 1, 10, 50 and 100 mW/g. These specific absorption rates (SAR) had no effect on the activity of either enzyme when the temperatures of the control and exposed sample mixtures were similar. These data demonstrate that the activity of these two enzymes is not affected by microwave radiation at the SAR's and frequency employed in this study.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment are of interest to the National Institute of Environmental Health Sciences. Before an accurate

evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control the temperature of the specimen carefully and be able to reproduce the exposure conditions. We have developed the capability to do this for in vitro exposures at NIEHS and this may provide a system for differentiating specific microwave effects from thermal responses. In addition, by examining the response of cells and cellular components to microwave radiation, it should be easier to identify the mechanism of action of microwave radiation with biological specimens.

PUBLICATIONS

Galvin, M.J., D.I. McRee and D.L. Parks: Microwave Irradiation and in vitro release of enzymes from hepatic lysosomes. Rad. Environm. Biophysic. 18: 129-136, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50061-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Oxidation of Ascorbic Acid During Histamine Secretion

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

PI: Mary J. Ortner Senior Staff Fellow LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Histamine secretion from rat mast cells is accompanied by the oxidation of endogenous ascorbic acid to the free radical species. Furthermore, although their existence has been shown biochemically, free radicals could not be trapped during histamine secretion. The generation of oxidized ascorbic acid during secretion, however, suggests that this anti-oxidant may function as a radical scavenger, thereby preventing the trapping of any generated radicals. It is postulated that the relatively high level of ascorbic acid in mast cells serves to protect the cell membrane from oxidative damage. Ascorbic acid may, therefore, contribute to the survival of mast cells after an extensive secretory response.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Although rat peritoneal mast cells have been studied extensively as a model system for the secretory response, the precise molecular mechanism of secretory stimulation is unknown. Indirect biochemical evidence has suggested, however, that superoxide and probably other radicals are generated by rat mast cells during either chemical or reaginic stimulation. It was logical, therefore, to attempt a more direct demonstration and identification of these radicals using spin trapping. This technique has successfully demonstrated the free radicals produced during the intense metabolic activity accompanying neutrophil phagocytosis.

Purified rat peritoneal mast cells were stimulated with compound 48/80 (a condensation product of p-methoxy-N-methyl phenethylamine and formaldehyde) in the presence of the spin-traps: PBN, phenyl-t-butyl nitrone, MNP, 2-methyl-2-nitrosopropane, or DMPO, 5, 5-dimethyl-1-pyrroline-N-oxide. The ESR spectra of the generated radicals were recorded using a Varian E-109 X-band spectrometer equipped with a TM_{110} cavity and a variable temperature accessory.

MAJOR FINDINGS AND PROPOSED COURSE: Histamine release experiments have shown that none of the spin traps used inhibited the secretory stimulation by 48/80 when added simultaneously. Although a very high instrument gain and a fast scan speed were used, there was no evidence to suggest that the radicals generated by 48/80 induced histamine secretion were trapped by any of the spin-traps used. A doublet separated by 1.7 Gauss, however, appeared immediately after the addition of 48/80 (20 μg/ml final concentration to 2 x 10 mast cells). Similar results were obtained in the absence of spin traps. In other experiments, purified mast cells were first treated with the spin trap MNP and 48/80 at 2°C, transferred immediately to a cold ESR cell and inserted into the cavity at 2°C. The ascorbic acid signal was generated between 10-20°C (when degranulation occurs) and disappeared above 30°C. There was again no evidence for the presence of trapped free radicals. In all of these experiments degranulation was confirmed microscopically after the spectra had been recorded. No radicals were generated by 48/80 addition to erythrocyte ghosts, mastocytoma cells, mast cell depleted peritoneal cells or heat-treated mast cells, none of which respond to this reagent.

Mast cells contain an unusually high concentration of ascorbic acid, which is known to scavenge many forms of reactive oxygen generated during intense metabolic activity. The appearance of ascorbic acid free radical in 48/80 stimulated mast cells indicates that it probably results from the rapid destruction of at least some of the primary radicals produced during histamine secretion. This may explain the failure to trap and identify the primary radicals known to be generated by the secretory process. Since these radicals are presumably reduced forms of oxygen, their generation during histamine secretion would be harmful to the cell unless they are rapidly destroyed. It is concluded that intrinsic ascorbic acid may serve as a protection against oxidative membrane damage following secretion in mast cells. This protection could improve the ability of mast cells to withstand massive degranulation with minimal damage to the membrane lipids and proteins. The cells would, therefore, be more likely to survive intact, resynthesize new granules, and restore their histamine secretory ability.

This project was completed in FY 80 but there has been a publication completed since that time (see PUBLICATIONS).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunological stimulation of histamine secretion via IgE antibody-antigen complex formation mediates the symptoms of asthma, hay fever and anaphylactic shock. In addition to this reaginic response, histamine secretion can also be initiated by several drugs and chemicals. Morphine, curare, chlorpromazine, concanavalin A, dextran, calcium ionaphores and 48/80 are among the many agents in this category. Histamine secretion by non-immunological means, therefore, presents a serious health hazard to those who are exposed to such drugs for therapeutic reasons. Mast cells are also implicated in the inflammatory reactions associated with photoallergic and phototoxic dermatitis. In addition, they may also exacerbate the conjunctivitis and pulmonary distress caused by certain xenobiotics in the atmosphere. The secretion of histamine by mast cells is preceded by extensive membrane fusion and is accompanied by increased oxygen consumption and the generation of free radicals. There are also dramatic alterations in mast cell biochemistry during the secretory response; although it remains unclear whether these changes reflect the metabolic pathway leading to membrane fusion or are a consequence of the membrane molecular rearrangement which has taken place. With the aid of biophysical techniques and compound 48/80, we are studying the molecular mechanism of histamine secretion. Theseudies may lead to the development of a safe method of control over both immunologically and environmentally related diseases due to histamine secretion by mast cells.

PUBLICATIONS

Ortner, M.J.: The oxidation of endogenous ascrobic acid during histamine secretion by rat peritoneal mast cells. Exp. Cell Res. 129: 485, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50062-02 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Enzymatic Reduction of C-Nitroso Compound

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

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The reduction of C-nitroso compounds such as nitrosobenzene and 2-methyl-2-nitrosopropane to nitroxide free radicals will be investigated. Although nitroso compounds are important reduction products of the more numerous nitro compounds, the enzymatic reduction of these compounds has received little attention. The biochemical implications of the reactions of the nitroxide intermediates will also be investigated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The enzymatic reduction of 2-methyl-2-nitrosopropane results in a four-line spectrum due to t-butyl hydronitroxide. The concentration of this free radical increased for over 30 min. This free radical accumulated in the presence of catalase (30,000 units/ml), but not in the presence of superoxide dismutase (30 μg/ml). Inhibition by superoxide dismutase is consistent with superoxide oxidation of the t-butyl hydroxylamine reduction product or reduction of the 2-methyl-2-nitrosopropane by superoxide.

Nitrosobenzene is known to be reduced by ascorbate to a species which will reduce ferricytochrome c. Other investigators have proposed this species to be an oxygen-reactive hydroxylamine radical R-NOH.

$$R\text{-}N = O + AH \longrightarrow R\text{-}\dot{N}\text{-}OH + A^{\cdot}$$

$$R\text{-}\dot{N}\text{-}OH + O_2 \longrightarrow R\text{-}N = O + O_2^{\cdot-}$$

A nitroxide free radical is more likely to be the species formed as can be confirmed by ESR.

$$R\text{-}N = O + AH^- \longrightarrow R\text{-}\overset{O}{N}H + A^{\cdot}$$

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.

$$AH^- + R\text{-}\overset{O.}{N}H \longrightarrow AH^{\cdot} + R\text{-}\overset{H}{\underset{O}{N}}H$$

$$R\text{-}\overset{H}{\underset{O}{N}}H + O_2 \longrightarrow R\text{-}\overset{O.}{N}H + O_2^{\cdot-}$$

Although it is well known that ascorbate can reduce nitroxides to form hydroxylamines, the reduction of nitroso compounds by either cofactors or enzymes to form hydronitroxides has not been reported.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 4-Nitroso-quinoline-N-oxide, 2-nitrosoflurene and 2-nitroso-2-naphthanol are a few of the nitroso compounds proposed to be ultimate carcinogens derived from the corresponding nitro compounds. Although the nitroxides are probably not DNA alkylating agents, they are probably intermediates in the formation of such species.

PUBLICATIONS

Kalyanaraman, B., Perez-Reyes, E. and Mason, R.P.: The reduction of nitroso spin raps in chemical and biochemical systems. A cautionary note. Tetrahedron Letters 50: 4809-4812, 1979.

Mottley, C., Kalyanaraman, B. and Mason, R.P.: Spin trapping artifacts due to the reduction of nitroso spin traps. FEBS Letters (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50063-01 LEB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biological Effects of High Pressure Sodium Vapor Lamps

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

PI: Colin F. Chignell Chief LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Sprague Dawley rats were born and reared under either daylight-simulating fluorescent lights or high pressure sodium vapor (HPSV) lamps. The total irradiance was 425 $\mu W/cm^2$ under both luminaires. The adrenal weights of both male and female rats housed under the HPSV lights were significantly higher than those reared under the daylight conditions. These results suggest that HPSV lamps, which have emission spectra that are grossly distorted when compared with natural daylight, may induce stress in rats. The significance of this finding to human health is unknown at the present time.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purpose of this study is to determine the effects of high pressure sodium vapor (HPSV) lamps on the development of the Sprague Dawley rat. Adult Sprague Dawley rats were paired and housed under either daylight simulating fluorescent lights or HPSV. The total irradiance of both lighting conditions was 425 $\mu W/cm^2$. The pregnant females were allowed to give birth and the litters were normalized to four males and four females soon after birth. The male rats in each litter were sacrificed at weaning (31 days of age) while the female rats were sacrificed when they were 71 days of age. The following weights were measured in each group: adrenals, kidneys, liver, heart, spleen and reproductive organs (testes or ovaries).

MAJOR FINDINGS AND PROPOSED COURSE: The weight of the adrenals was significantly higher ($p < 0.001$) in the male rats kept under the HPSV lamps compared to the daylight controls (12.8 mg/100g body weight vs 10.8 mg/100g body weight). The adrenals of the female rats exposed to the HPSV lamps were also significantly ($p < 0.001$)heavier than the controls (15.0 mg/100g body weight vs 13.8 mg/100g body weight). The only other difference noted was that the hearts of the male rats in the HPSV group were significantly ($p < 0.001$) heavier than the controls (486 mg/100g vs 450 mg/100g).

The observation that the adrenals of both the male and female rats reared under the HPSV lamps were significantly heavier than those of the control animals strongly suggests that the HPSV lamps are somehow subjecting the rats to stress. However it should be pointed out that, because rodents have only rods in their retinas, they will perceive differences in brightness between the daylight fluorescent lights and the HPSV lamps. Since rats are nocturnal creatures, which normally avoid brightly lighted environments, the observed effects may be due to non-specific stress rather than a specific effect arising from spectral differences. These experiments are therefore being repeated under illumination conditions which have been normalized for differences in perceived brightness.

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 schools and offices it is important to determine whether they produce any undesirable biological effects.

PUBLICATIONS

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50064-01 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Behavorial Effects of Noise Exposure During Fetal Life

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

PI:	Hugh Tilson	Pharmacologist	LBT	NIEHS
	Reginald Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics/Lab. of Behaviorial & Neurological Tox

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Noise stress during pregnancy has been reported to be embryolethal, as well as teratogenic, in rats and mice. However there have been few studies concerning the possible effects of prenatal noise stress on the development of neurobehavioral functioning of the offspring; although a combination of light and heat stress has been reported to cause changes in catecholamine levels in brain areas affecting sexual differentiation of adult rats exposed as fetuses and to cause demasculinization of male offspring. The purpose of this experiment is to determine the effects of prenatal noise stress on neurological functioning and reactivity of offspring. The results of these experiments might be used in the planning of future studies that (1) compare the effects of noise stress to other stressers (2) determine the role of stress-induced release of catecholamines in the medication of any long-term behavioral effects and (3) measure those changes in neurochemical and neuroendocrinological status in stress exposed animals that may be correlated with any behavioral changes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: At least 16 sperm positive gravid females will be obtained from the in-house Fischer 344 rat breeding colony. The pregnant animals will be housed individually in single stainless steel cages (25 cm x 35 cm x 22 cm) contained within sound and light attenuating cubicles (IAC-400 sound booth) equipped with ventilation fans, temperature controls and automatic lighting system. Lights will be turned on at 0600 hrs and turned off at 1800 hrs. Food (NIH Lab Chow #30) and water will be freely avialable.

Maternal weights will be taken on days 5 and 21 of gestation. From day 7 to day 21 of gestation, 8 gravid rats will be selected on a random basis for exposure to an octave band of noise centered on the region of most acute auditory sensitivity of the rat (8 kHz) for 12 hr daily (1200 to 2400). The remaining 8 rats will be housed under similar conditions, but will be exposed to ambient levels of noise inside housing cubicles (≈ 20-30 dB). On the day of birth (day OPN), the birth weights, litter size, sex ratio of the litter will be determined. On day 1 postnatally (PN) the mothers and pups will be removed from Building 10 and taken to Building 2, Room 210. On day 4 PN, the litters will be culled to 4 male and 4 females. Body weight of all pups will be taken on days 4, 7, 14 and 21 PN. On day 21 PN, the pups will be weaned. One male and one female from each litter will be selected randomly for subsequent behavioral studies. The remaining animals will be discarded or used for neurochemical or neuroendocrinological assays.

Rats selected for future studies at weaning will be weighed, housed 4 per cage according to sex and treatment and ear punched for future identification (total N=8 litters x 2 treatments x 2 [sexes] = 32 rats). Rats will be assessed for 60 min motor activity @30 and 100 days PN. Primary Test Battery (PTB) measures and body weights will be taken on days 31 and 101 and will include: 1. Tail Flick - Procedural Protocol No. 093-BT. 2. Startle to acoustic stimulus/air puff - Procedural Protocol No. 083-BT. 3. Negative geotaxis - Procedural Protocol No. 084-BT. 4. Foe-/hindlimb grip strength - Procedural Protocol No. 074-BT.

Data from the Automex and PTB measures will be analyzed as described in their respective procedural protocols using a 2 (treatment) x 2 (sexes) x 2 (time points) analysis of variance (ANOVA). Maternal weight gain and litter measurements will be tested for treatment effects using normal parametric tests (t-tests). Preweaning body weights will be assessed using a 2 (treatments) x 2 (sexes) x 4 (time points) ANOVA; postweaning body weights will be assessed using a 2 x 2 x 2 ANOVA.

MAJOR FINDINGS AND PROPOSED COURSE: Accomplishments to date consist of development of a compact animal exposure system where large numbers of rats can be exposed in a uniform, high level sound field without disturbing other lab experiments in progress. These requirments led LEB personnel to design and construct a unique exposure chamber with compact all-wire cages, automatic watering and temperature control.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Findings indicating that maternal light and heat stress may lead to permanent brain catecholamine level differences and produce overt behavioral (demasculimization) changes in offspring suggest that other overt behavioral change may also be produced. Since noise is a much more prevasive stressor in modern society than light or heat, its effects should be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50065-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Superoxide and Lipid Peroxidation in Biological Properties of Anthracyclines and DHAQ

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

PI: Birandra K. Sinha Senior Staff Fellow LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The biochemical properties (antitumor, carcinogenic and cardiotoxicity) of the anthracyclines may be related to DNA binding and or the production of oxygen derived toxic free radical metabolites. We have examined the formation of O_2^- from these drugs and have evaluated the role of oxygen derived toxic species in their biological properties. Our findings indicate that formation of O_2^- is not related to the antitumor activity. However, lipid peroxidation caused by O_2^- or $OH^\cdot$ is related to the cardiotoxicity of the anthracyclines.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal-NADPH incubations of both adriamycin and daunomycin have been shown to generate semiquinone free radical intermediates which react with oxygen to form superoxide ($\dot{O}_2^-$). The semiquinone free radical or $\dot{O}_2^-$ or its toxic metabolites (OH/H_2O_2) have been recently proposed to act as primary cytotoxic agents. In addition, O_2^- and OH/H_2O_2 have been implicated in anthracycline-induced cardiotoxicity through lipid peroxidation of the cardiac cell membranes.

MAJOR FINDINGS AND PROPOSED COURSE: The ability of six anthracyclines and 1,4-bis-{2-[(2-hydroxyethyl)amino]ethylamino}-anthracenedione (DHAQ), with demonstrated antitumor activity and cardiotoxicity, to stimulate $\dot{O}_2^-$ production in rat hepatic microsomes and beef heart submitochondrial particles has been studied. Our findings indicate that the semiquinone free radical is formed in heart submitochrondrial particles which stimulates the production of $\dot{O}_2^-$ in a manner similar to that seen with rat hepatic microsomes. While daunomycin stimulated a large production of $\dot{O}_2^-$ in rat hepatic microsomes, the most active antitumor agents, DHAQ and AD-32, stimulated significantly smaller production of $\dot{O}_2^-$. This would suggest that the antitumor activity may not be related to $\dot{O}_2^-$ formation. In addition, fully intercalated drugs are not effective in $\dot{O}_2^-$ production and hence cannot act as carriers for site specific production of $\dot{O}_2^-$.

Our studies also show that these agents also induce peroxidation of rat hepatic microsomal lipid. Agents which are highly cardiotoxic (adriamycin, daunomycin and N, N-dimethyl daunomycin) induce significantly more lipid peroxidation than those agents which are less cardiotoxic (AD-32, rubidazone, carminomycin and DHAQ). Thus it appears that lipid peroxidation plays an important role in the pathogenesis of cardiotoxicity. Furthermore, α-tocopherol, a known free radical scavenger, afforded only a marginal protection against lipid peroxidation induced by these agents. Reduced glutathione, on the other hand, afforded a complete protection agains lipid peroxidation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since oxygen derived toxic free radical metabolites have been implicated in carcinogenesis, cytotoxicity, and cardiotoxicity of a number of chemicals, it is of great interest to understand the molecular mechanism of $\dot{O}_2^-$ ($OH;H_2O$) formation and their relevance in the expression of toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50066-01 LEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structure and Reactions of Free Radicals from Serotonin and Related Indoles

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

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The ESR spectra of the free radicals formed by the autoxidation of serotonin, 5-hydroxyindole, and 5-hydroxytryptophan in 1 N NaOH have been detected. The analysis of the hyperfine splitting constants in H_2O and D_2O characterize these free radicals as semiquinone-iminies, the one-electron oxidation product of the corresponding indole. At alkaline pH, autoxidation of these compounds ultimately leads to solid precipitate and unresolved ESR spectra characteristic of polymeric material. The reduction of cytochrome *c* at pH 7.4 by a wide variety of indoles correlates with the amplitude of the ESR signal in 1 N NaOH, as do other processes though to be related to 5-hydroxyindole free radical formation. Relative to the rate of cytochrome *c* reduction, neither serotonin nor the serotonin free radical appears to react with oxygen to form superoxide. In the presence of NAD(P)H, the serotonin radical most probably oxidizes NAD(P)H to form the NAD(P)$^{\cdot}$ radical. The NAD(P)$^{\cdot}$ radical then reacts with oxygen to form superoxide, which ultimately reduces cytochrome *c*.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: A serotonin free radical was observed by Borg in 1964 using electron spin resonance. The radical was generated during permanganate oxidation in alkaline solution ($KMnO_4$, 0.1 N NaOH); however, the biphasic decay kinetics suggested the presence of two free radical species. Due to the importance of serotonin, we decided to reinvestigate the ESR spectrum of its free radical. In addition, we have studied the ESR spectra of 5-hydroxy-tryptophan and 5-hydroxyindole using deuterium isotope substitution and, where possible, an ESR resolution enhancement technique of 90° out-of-phase dection.

The history of the serotonin radical begins in 1961 when Walaas and Walaas presented spectrophotometric evidence that ceruloplasmin could generate the radical, which then oxidized reduced pyridine nucleotides. Later, Alivisatos and Williams-Ashman reported the synergistic stimulation of cytochrome *c* reduction by serotonin and NADH. The mechanism of this stimulation was further studied under anaerobic conditions by Polacow and Cilento. They postulated that serotonin made cytochrome *c* more susceptible to reduction by NADH, without the formation of the serotonin radical. We have reinvestigated this system under aerobic conditions and report the effect of superoxide dismutase and catalase. Our ESR and cytochrome *c* reduction results demonstrate the importance of free radical reactions of serotonin and other 5-hydroxyindoles.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Free radical reactions of the serotonin semiquinone-imine free radical with itself, serotonin, serotonin quinone-imine, and protein may be important in the formation of this melanin-like material. Whether 5-hydroxyindole-derived melanin actually forms in the brain is unknown, but *in vitro* experiments clearly indicate that the one-electron oxidation of serotonin is possible under physiological conditions.

PUBLICATIONS

Perez-Reyes, E. and R.P. Mason: Characterization of the structure and reactions of free radicals from serotonin and related indoles. J. Biol. Chem. 256: 2427-2432, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50067-01 LEB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Studies of Anthracene Phototoxicity in Rat Mast Cells and Human Erythrocytes

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

PI: Mary J. Ortner

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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 (200 words or less - underline keywords)
Anthracene, a component of coal tar, has proven to be a very potent photosensitizer in human erythrocyte ghosts. Anthracene was bound covalently to different positions on fatty acid molecules. Phototoxicity studies showed that when anthrace was bound to the polar end, it was more photoxic than anthracene alone. However when bound to the apolar end, anthracene had little phototoxic effects. The major site of anthracene phototoxic reactions therefore appear to be close to the protein-lipid interface within the ghost membranes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: The light-excited triplet state of endogenous photosensitizers (e.g. protoporphyrin) or phototoxic drugs (e.g. tetracycline) initiates oxidative reactions within erythrocyte membranes that ultimately lead to membrane damage and hemolysis. The most damaging molecular reaction is probably intermolecular protein crosslinking caused by covalent bond formation between free amine groups and photooxidized histidine residues. Although the generation of active oxygen (e.g. O_2^-, $^1O_2$) has been implicated as an intermediary in this reaction, the primary molecular mechanism of phototoxic reactions is unknown.

Anthracene, a polycyclic aromatic hydrocarbon component of coal tar, is currently being studied. This environmental pollutant is a potent photosensitizer and may prove useful in determining the molecular mechanism of light-induced toxic reactions.

METHODS EMPLOYED: Human erythrocytes are being used to study the phototoxic effects of anthracene on cell lysis and on isolated membrane proteins. In addition, rat peritoneal mast cells are being used to study phototoxic reactions in a functioning secretory cell. Standard illumination techniques are employed and circular dichroism spectroscopy has also been developed with the objective of identifying the molecular species directly involved in protein photodegradation.

MAJOR FINDINGS AND PROPOSED COURSE: Erythrocytes exposed to anthracene show up to 78% hemolysis after 90 min illumination. The effect is concentration and time dependent and does not occur in the dark. Recent experiments using anthracene-derivatized fatty acids have shown that anthracene is much more toxic to erythrocytes when covalently bound close to the polar end of the fatty acid molecule (2-anthroyl-palmitic acid). Anthracene attached to the 16th carbon of palmitic acid was not effective as a photosensitizer. These data indicate that the site of photosensitization by anthracene is near the interface region of the erythrocyte membrane. Future plans include circular dichroism studies of D_2O. The phototoxic properties of anthracene and anthracene-derivatized fatty acids will also be investigated using the rat peritoneal mast cell system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Coal tar derivatives constitute a significant contribution to environmental pollution. It is important therefore to understand the mechanism of anthracene toxicity, especially in relation to a possible synergistic effect with sunlight. The present study is currently evaluating the effects of anthracene in both erythrocytes and secretory cells. Since very little is known about the phototoxic effects of this compound, we are also studying its molecular mechanism and its site of action in the membrane. These studies may also prove useful in the clarification of phototoxic mechanisms in general.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 50068-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on Acute in vivo Exposure of Rats to 2450 MHz Microwave Radiation

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

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation

INSTITUTE AND LOCATION
NIEHS, NIH, Research Traingle 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 (200 words or less - underline keywords)

Acute 8 hour exposure of rats to 2450 MHz (CW) microwave radiation was performed at power levels that cause no increase in colonic temperature. There were no measurable changes in the mast cell response to compound 48/80 or to concanavalin A, nor were any morphological changes observed. There was no change in the thyroid axis, however alterations were seen in serum corticosterone levels in the 10 mW/g (SAR) group. No change in hematocrits or erythrocyte number was observed and there were no changes in total leukocytes, or in the numbers of lymphocytes, neutrophils, and monocytes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Acute exposure to 2450 MHz microwaves radiation in the rat has been studied previously for varying periods of time. Although high power densities cause significant biological changes, these alterations are usually accompanied by substantial increases in colonic temperature. However, several studies have reported microwave-induced changes in immunocompetence, hematopoiesis, cytological parameters and neuroendocrine secretion at incident power densities of 20 mW/cm^2 or less. Under these conditions, there was no apparent temperature increase and the biological effects have been attributed to other putative microwave characteristics.

We have studied the effects of acute exposure at two power densities on several systems in the rat. Peritoneal mast cells store several potent vasoactive agents (e.g. histamine). Membrane activation through several distinct pathways can cause active secretion of these factors and their consequent physiological effects. Mast cells are thus responsive to a wide variety of external stimuli and their secretory ability is influenced by several biochemical and physioloigcal parameters. The possibility therefore exists to demonstrate both primary and secondary microwave effects in vitro.

The mammalian hypothalamo-hypophyseal-thyroid (HHT) axis and hypothalamo-hypophyseal-adrenal (HHA) axis of the endocrine system regulate body functions that maintain homeostasis in basal and stress states. The functional competence of these axes is essential to survival of the animal within a changing environment. Since microwave exposure can be considered as a type of environmental stress, its effects on the activities of the HHT and HHA axes are important.

Hematologic and biochemical effects of microwave radiation have been reported including a significant leukopenia, lymphopenia and neutrophilia, and significant increases in blood glucose, cholesterol and glutamic oxaloacetic transferase activity. These data suggest that blood composition (hematology and biochemistry) is sensitive to microwave radiation.

METHODS EMPLOYED: Exposures were carried out within an environmental chamber. Both control and exposed animals were housed individually in styrofoam cages. The animals were in positions which received similar field intensities. The incident power density was determined directly using NBS Model B probe inserted in a sample cage with and without animals in the field. Each experimental group consisted of 8 male rats (Charles River, CD) with weights of 300 $\pm$ 35 g. Twelve experiments (4 control, 4 low and 4 high power exposures) were run on consecutive days alternating the control, low power density (2 mW/cm^2) and high power density (10 mW/cm^2) groups. The control rats were treated exactly as the exposed ones except that the power was not turned on. The animals were weighed immediately before and after exposure. The animals were irradiated for 8 hrs with food and water withheld to prevent aberrations in the field. Within 5-15 min after irradiation, six animals were decapitated, the peritoneal mast cells extracted in Lockes solution, and blood was removed for hematology and endocrinology determinations. The two remaining animals were immediately anesthetized with pentobarbital and the aorta cannulated for blood pressure determinations.

MAJOR FINDINGS AND PROPOSED COURSE: The data on wave penetration in biological tissue indicate that under our exposure conditions, the blood basophils, the cutaneous mast cells and probably most of the peritoneal mast cells received direct microwave exposure. In addition, some conductive heating probably continued beyond the actual depth of pentration (approximately 2 cm). Nevertheless, the viability studies showed that this potential stressor did not greatly damage the cells of the peritoneal cavity or disturb the normal basophil count in the blood.

We have studied the performance of irradiated mast cells stimulated via the chemical (48/80) and the immunological (Con A) pathways. Compound 48/80 is one of the most potent and efficacious histamine liberators and it acts by stimulating putative receptors on the cell membrane. Con A, in contrast, stimulates the cell by crosslinking membrane-bound IgE molecules in a manner similar to antigen-antibody interactions. The proper functioning of both pathways depends on cellular energy metabolism, cyclic nucleotides, cytoskeletal elements and in the case of Con A, calcium transport. The present in vivo irradiation data now confirm our previous findings in vitro which showed that microwave radiation did not affect the complicated secretory pathways of these activators. Furthermore, we have shown that after intraveous injection of 48/80, the in vivo secretory response of the basophils and mast cells was unimpaired. This indicates that these cells are not adversely affected by any secondary factors potentially brought about by the radiation (e.g. release of hormones, neurotransmitters, etc.).

Serum concentrations of T_4 and T_3 were not altered by the experimental conditions used. T_3-uptake values, representing the unsaturated binding capacity of thyroid binding globulin, were similarly unaffected. FTI and AT_4 values, which are calculated from the T_3-uptake data and are proportional to free T_4 levels, did not exhibit significant differences between treatment groups. Rats exposed for 8 hours to 0 mW/cm^2 or 2 mW/cm^2 had higher serum corticosterone levels than did untreated control rats. Those exposed to 10 mW/cm^2 did not exhibit an increase as did the 0 and 2 mW/cm^2 groups, but rather had values similar to the untreated controls.

The level serum protein in general and of β-glucuronidase, alkaline phosphatase, catic dehydrogenase and cholinesterase in particular was not altered by microwave exposure at either power density. In addition, no effects of microwave radiation were noted on sodium and potassium levels. No change in the hematocrit or RBC number was noted for either exposure group, furthermore, there were no changes in the total number of ciruclating white blood cells, or in the precentage of white cell types, including lymphocytes, neutrophils, or monocytes.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent advances in microwave technology have resulted in increased potential for exposure of both the general public and technical personnel to non-ionizing radiation. Because the biological consequences of acute exposure to alternating electromagnetic fields are largely undefined, investigations utilizing microwave radiation are of current interest. The present studies have shown that acute exposure to microwave radiation does have significant effects on certain endocrine parameters, whereas other systems are unaffected. These results suggest that there

are target systems that are especially sensitive to microwave-induced stress. This project has been completed.

PUBLICATIONS

Ortner, M.J., M.J. Galvin and D.I. McRee: Studies on acute in vivo exposure of rats to 2450 MHz microwave radiation. I. Mast Cells and Basophils. Rad. Res. (In press).

Abhold, R.H., M.J. Ortner, M.J. Galvin and D.I. McRee: Studies on acute in vivo exposure of rats to 2450 MHz microwave radiation II. Effects on thyroid and adrenal axis hormones. Rad. Res. (accepted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50069-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biophysical Studies on the Effects of 2450 MHz Microwave Radiation

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

PI:	Mary J. Ortner	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Richard Irwin	Chemical Manager	TRTP	NIEHS

COOPERATING UNITS (if any)

Toxicology Research and Testing Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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 (200 words or less - underline keywords)

Instrumentation has been developed for the study of fluorescence or circular dichroism during exposure of biological samples to microwave radiation (2450 MHz, CW). Fluorescence experiments have shown that microwave radiation at SAR's of 10 and 200 mW/g had no effect on calcium binding to human erythrocyte membranes or on energy transfer between membrane bound probes and intrinsic tryptophan residues. Circular dichroism measurments have shown that spectrin molecules from human erythrocyte membranes may be affected by high microwave power levels (600 mW/g, SAR). These effects may result from differential intramolecular interactions with the oscillating electric field. Experiments utilizing these instruments will help to clarify reported microwave effects by examining them on a molecular basis.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: Microwave induced alterations in animal physiology must eventually be defined in terms of cellular pathophysiology and ultimately on a molecular level. We have therefore developed two biophysical instruments that interface precisely with sample chambers to provide accurate microwave exposure, dosimetry and temperature control. Molecular studies of drug binding and membrane protein conformation can thus be conducted before, during and after exposure of a sample to microwave radiation.

METHODS EMPLOYED: The effect of 2450 MHz microwave radiation on the proteins of human erythrocyte ghosts has been investigated using ultraviolet circular dichroism spectroscopy. A specially constructed waveguide inserted into a spectropolarimeter allowed continuous recording of the optical activity due to secondary structure in membrane proteins. Microwave-induced conformational changes in protein α-helical structure could then be compared with the effects of conventional heating.

The effect of 2450 MHz microwave radiation on erythrocyte membrane protein conformation and calcium binding was studied with the fluorescent probe, 1-anilino-8-naphthalenesulfonate (ANS). Using fiber optic cables, excitation light was delivered to a stirred sample undergoing irradiation (2450 MHz, CW) within a fluid-filled, temperature-controlled waveguide. Fluorescence was collected using an identical cable and transferred through appropriate filters to standard detecting, amplificaton and recording devices.

MAJOR FINDINGS AND PROPOSED COURSE: The ultraviolet circular dichroism data indicate that high levels of microwave radiation (600 mW/g, specific absorption rate) induce decreases in α-helical conformation that may be due both to thermal vibrations and to increased strain on the intramolecular hydrogen bonds that maintain secondary structure. The latter effect may result from differential intramolecular interactions with the oscillating electric field. Spectrin (bands 1 and 2) isolated from the ghosts was more sensitive to microwave irradition than intact ghosts, and spectrin-depleted vesicles were the least sensitive. The data, therefore, indicate that the α-helical conformation of spectrin is altered by high levels of microwave radiation.

Microwave radiation at specific absorption rates of 10 and 200 mW/g had no effect on the binding of the fluorescent probe, ANS, to the erythrocyte membranes. Concentration response curves of increased fluorescence intensity versus calcium concentration also showed no microwave influence on calcium binding between 2.0 - 10.0 x 10^{-4}M. In addition, experments studying fluorescence energy transfer between intrinsic tryptophan residues and membrane bound ANS showed that intermolecular distances between donor and acceptor were also unaffected by microwave radiation.

These specially developed instruments are being used to investigate possible molecular mechanisms responsisble for the many putative effects of microwave radiation, including possible synergistic effects. Both circular dichroism and the fluorescence detecting instrumentation are now being used to study effects of simultaneous microwave exposure on the polymerization of microtubules and specific drug binding (e.g. colchicine, vinblastine) to tublin.

The effect of microwave radiation on brain mitochondrial membranes is also being investigated using ANS, which is sensitive to changes in charge distribution due to substrate (e.g succinate, NADH) oxidations. Because of the evidence supporting a specific microwave effect on embryogeny, circular dichroism spectroscopy will be used to study microwave effects on both intact chromatin and isolated DNA. Chromatin will be studied using intact avian erythrocyte nuclei. Circular dichroism will also be useful to study the effects of microwaves on drug binding to DNA, cell membranes and subcellular organelles. Future fluorescence projects include a study of the reported synergism between chlordiazepoxide and microwaves. The effects of irradiation during the binding of this and other neuropharmacological drugs to isolated brain synaptosomal membranes will be studied. Microwave effects on fertility have been demonstrated in this laboratory after irradiation of avian sperm. The effect of microwaves on sperm membrane protein sulfhydryl groups and sperm-tail microtubular protein will therefore also be studied using fluorescent probes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent advances in microwave technology have resulted in increased potential for exposure of both the general public and technical personnel to non-ionizing radiation. Because the biological consequences of acute or chronic exposure to alternating electromagnetic fields are largely underfined, investigations utilizing microwave radiation are of current interest. Teratological, neurological, immunological and hematological alterations in both avian and mammalian species have been ascribed to a putative "non-thermal" or "electromagnetic" stress following whole body exposure to microwave radiation. In addition, recent reports have suggested that low level exposure to microwaves may also sensitize animals to the effects of drugs in a synergistic manner, presumably by inducing an occult stress. Unfortunately, the data are often contradictory due to a wide variation in experimental techniques, frequencies and animal models and the subtle nature of the putative effects. As a result, a unifying mechanistic concept to explain the biological effects of microwave radiation has not been developed.

In order to understand the biological effects of microwave radiation, the - alterations in normal physiology must eventually be defined in terms of cellular pathophysiology and ultimately at the molecular level. The development of the present methodology will aid in explaining many of the putative effects of microwave radiation.

PUBLICATIONS

Ortner, M.J., M.J. Galvin, C.F. Chignell and D.I. McRee: A circular dichroism study of human erythrocyte ghost proteins during exposure to 2450 MHz microwave radiation. Cell Biophysics, (accepted).

Ortner, M.J., M.J. Galvin, D.I. McRee and C.F. Chignell: A novel method for the study of fluorescent probes in biological material during exposure to microwave radiation. J. Biochem. Biophys. Meth. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50070-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Different Noise Exposures on Embryo/fetal Development in the Mouse

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

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
OTHER:	Peter S. Nawrot	Visiting Associate	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

In previous work (see Z01 ES 50044-02) designed to evaluate the reproductive and teratogenic effects of exposure to different noise spectra and temporal patterns, one of the spectra was associated with an increase in late stage fetolethality in the CF-1 mouse. This narrow band 18-20 kHz spectra, produced by a commerical device alleged to be aversive to rodents, was cycled on a one second rate from noon to midnight. In order to determine whether the effect was solely related to acoustic energy in the most sensitive auditory frequency band of the mouse (15-30 KHz) or to some other unknown factor, an experiment involving a band spanning 10-45 kHz was employed. Unfortunately CF-1 mice were no longer available to us; CD-1 mice were employed in this experiment.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Methods duplicated those described previously (Z01 ES 50044-02) except for change in noise spectra and mouse strain. Briefly, three groups were utilized. One group was exposed on days 1-6 of gestation, (preimplantation) a second group on days 6-15 of gestation, and a third group served as control. On day 18 of gestation females of all experimental and control groups were sacrificed by cervical dislocation and their reproductive status was determined. The uterus was exposed and the number of implantation sites counted. The conceptus at each site was examined and classified as resorbed, dead or alive. The full-term fetuses (live and dead) were individually weighed, the sex determined, and examined for external malformations. Any fetus weight 0.5 g or less or any live fetus weighing less than two-thirds the average of its largest litter mate was termed stunted. At least one-third of the fetuses of each litter were examined for visceral alterations. In addition, all stunted fetuses and those with external malformations were examined for visceral alterations. All live fetuses at time of sacrifice were processed and examined for skeletal alterations.

MAJOR FINDINGS AND PROPOSED COURSE: Decreases in pregnancy maintainence, fetal weight, and late stage fetal death were demonstrated but the significance of each was much lower than found in the previous experiment where CF-1 mice were exposed to a narrow band of temporally cycled noise. Since these results indicate that the mere presence of high level noise in the most sensitive auditory band of the mouse is not a sufficient conditon for late stage fetolethality, the effect of species difference should be investigated. In addition the period of exposure should be extended from the 15th day to near parturition, since catecholamine level changes play a major role in this event.

SIGNFICIANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been voiced regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. Several epidemiological studies around airports have reported low level increases in teratogenicity. Our findings indicate that at least in the mouse, teratogenicity resulting from excessive noise exposure is very difficult to induce. Other reproductive aberrations appear to be much more likely.

PUBLICATIONS

Nawrot, P., Cook, R.O. and Hamm, C.W.: Embryotoxicity of broad band high frequency noise in the CD-1 Mouse. J. Tox. and Envir. Health. June 1981 (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50071-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Electro Acoustic Factors Affecting Speech Discrimination

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

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
OTHER:	W.G. Thomas	Director, Hearing and Speech Program	N.C. Memorial Hospital	
	Blake Wilson	Senior Electrical Engineer	RTI	

COOPERATING UNITS (if any)

N.C. Memorial Hospital and RTI

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A line of investigation which began with the limited purpose of comparing the discriminability of speech mechanically coupled to the ossicular chain with similar speech passed through hearing aids, led to an awareness that the manner in which speech discrimination formulations were conventionally utilized failed to take into account important electro acoustic variables. Pilot tests suggested that failure to account for these variables might be a prime factor in the continuing controversy regarding the precision of phonetically balanced monosyllablic word lists, the most widely used speech discrimination formulation. Attempts to systematically elucidate the relevant parameters were initially focused on the method of "equalizing" speech made dissimilar in speech peak to long term RMS level by amplitude distortion. Discrimination scores obtained by listeners from lists which had been equalized on the basis of their long term RMS level showed that this method was superior to conventional peak equalization methodologies in preserving the intrinsic discrimination superiority of less distorted speech.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electronic measurement systems were utilized to precisely assess, analyze, and compare electro-acoustic parameters of conventional speech formulations which had been deliberately made unequal by passage through differentially distorting transducers. Listener test scores were obtained to determine the intelligibility of the resulting speech when equalized according to long term RMS level and the conventional peak method.

MAJOR FINDINGS AND PROPOSED COURSE: In discrimination tests where electro-acoustic amplitude parameters were systematically varied it was shown that confounding occured when the conventional peak equalization method was used to equalize significantly distorted speech material with nondistorted or lightly distorted speech, obviating the intrinsic discrimination superiority of the latter. When the different materials were equalized on the basis of total energy content, listener tests showed that the intrinsic discrimination superiority of the less distorted speech was preserved. By utilizing this technique it was also shown that speech mechanically coupled to the ossicular chain of guinea pigs and recovered from the inner ear by differential electrode techniques, was discriminated equally with speech which was acoustically coupled, but was discriminated significantly better than speech passed through conventional hearing aids. In addition, an electronic device which will make it possible for others to test a variety of equalization procedures was designed and fabricated.

Techniques validated as a result of the above investigations will be employed in recording new test tapes designed to (1) determined whether speech spectrum noise and babble of equal spectral density are equally efficient speech maskers, (2) determine the effect of matching signal and competition spectrum on discrimination test results, and (3) determine whether speech discrimination differences which may occur when competion and speech signal are passed through hearing aids separately and simultaneously correlate with hearing aid intermodulation distortion characteristics. The role of the Noise Effects Group will be limited to preparing the tapes, others will acquire the listeners and perform the actual testing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Scientifically validated procedures are needed for assessing the degree of handicap for speech discrimination associated with varying amounts of pure tone hearing loss and for objectively relating hearing aid characteristics to hearing loss characteristics. Without well validated procedures it is difficult, if not impossible, to assess the costs associated with the social and economic impairment suffered by our 16 million hearing impaired citizens. Without realistic hearing loss cost figures, the benefits associated with hearing conversation-noise reduction programs cannot be calculated, making cost-benefit decisions highly subjective.

PUBLICATIONS

Cook, R.O., Hamm, C.W., Thomas, W.G. and Royster, L.W.: Comparison of acoustically coupled and direct ossicular chain coupled speech. Audiology 20: 00-00, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50072-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ototoxicity of Cis-dichlorodiammine Platinum

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Bhola N. Gupta	Pathologist	TRTP	NIEHS

COOPERATING UNITS (if any)

Toxicology Research & Testing Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Cis-dichlorodiammine platinum, an agent with antineoplatic activity, was examined by otototic effects in guinea pigs. Ototoxicity was evaluated by suppression of the cochlear potentials, alteration of electrolyte concentrations in the cochlear fluids and by histopathological changes in the inner ear. Guinea pigs treated with ip injection of cis-dichlorodiammine platinum 1.5 mg/kg (5 doses/wk) developed marked suppression of sound-evoked cochlear responses but showed little changes in the endocochlear potential. The electrolyte concentrations of the cochlear fluids were not substantially altered in cis-dichlorodiammine platinum-treated guinea pigs.

PHS-6040
(Rev. 2-81)

PROJECT DISCRIPTION

METHODS EMPLOYED: Healthy guinea pigs were treated with daily ip injection of 1.5 mg/kg of cis-dichlorodiammine platinum five times a week. One week before the treatment a silver wire electrode was chronically implanted near the external ear canal under pentobarbital anesthesia. During treatment with cis-dichlorodiammine platinum electorcochleography was carried out twice a week. Treatment was continued until consistent suppression of the electrocochleograms. After an additional observation period of about 1 week animals were anesthetized with sodium pentobarbital and the sound evoked cochlear potentials and the endocochlear potential (EP) were recorded from the basal and third turn of the cochlea. The perilymph and endolymph were collected and Na^+, K^+ and Cl^- concentrations were determined. The temporal bones and kidneys were removed for histological examination. A group of healthy guinea pigs treated with daily ip injection of sterile water (1.5 ml/kg) was used as control animals. Additional normal guinea pigs without treatment were used to evaluate effects of treatment.

MAJOR FINDINGS AND PROPOSED COURSE: Data were obtained from 11 experimental guinea pigs, 3 control guinea pigs and 17 normal guinea pigs without treatment.

1. General conditions: General conditions in the platinum treated guinea pigs were not markedly deteriorated. The body weight tended to increase slightly. No vestibular disturbance was observed in any experimental animals.

2. Electrocochleography: The action potentials in response to unfiltered clicks recorded with the implanted electrode were stable during the first 2 weeks and progressive suppression of the action potentials occured during the 3rd and 4th week of the treatment. The action potential remained little changed during an additional one week observation period. The electrocochleography in control guinea pigs showed stable responses throughout the experimental periods.

3. Cochlear potentials: When compared with control animals, the plantinum-treated guinea pigs showed suppression of cochlear microphonics (CM) and action potential (AP). The mean loss of CM_{sens} (sound intensity required to elicit 100 μV peak-to-peak CM) and mean maximum output of CM in response to 6 kHz were 10 dB and 11 dB respectively. The CM in response to 500 HZ tone burst recorded from the third turn of the cochlea also showed a decrease in its magnitude in platinum-treated guinea pigs but to a less extent than suppression of CM in the basal turn. The mean values of EP recorded from the basal and third turn of the cochlea were 79.8 mV and 68.8 mV which were comparable with those obtained from the control guinea pigs.

4. Electrolyte concentrations in the cochlear fluids: Treatment with a cis-dichlorodiammine platinum did not result in marked changes in K^+, Na^+ and Cl^- concentrations in both endolymph and perilymph

	Platinum	Control
	$[K^+]$ in mM/l	
Perilymph (scala vestibuli)	5.4 ± 2.2	6.1 ± 1.5
Perilymph (scala tympani)	2.8 ± 0.6	3.1 ± 0.6
Endolymph	159.2 ± 4.3	157.1 ± 6.5
	$[Na^+]$ in mM/l	
Perilymph (SV)	156.6 ± 26	149.3 ± 11.3
Perilymph (ST)	150.9 ± 7.4	152.2 ± 13.7
Endolymph	0.6 ± 0.5	0.3 ± 0.3
	$[Cl^-]$ in mM/l	
Perilymph (SV)	122.9 ± 2.7	119.8 ± 5.5
Perilymph (ST)	121.6 ± 3.1	123.2 ± 4.1
Endolymph	132.6 ± 4.1	128.8 ± 3.3

We plan (1) to evaluate the histological lesions in the cochlea and correlate histological findings with the electrophysiological data and (2) to study possible histological changes in the kidney of the platinum treated guinea pigs and to correlate the renal dysfunction with the cochlear damages. The experimental data will be analyzed and a manuscript will be submitted for publication in the near future.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the ototoxicity of platinum has been reported, the pathogenesis of platinum-induced deafness have not been extensively studied. The present study will make a significant contribution for the understanding of ototoxicity of cis-dichloro-diammine platinum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50073-01 LEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of 2.45 GHz Microwave on Brain Development

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

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COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Enviornmental Biophysics

SECTION
Nonionizing Radiation Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Male Sprague-Dawley rats were exposed 50 2.45 GHz microwave, radiation at an incident power density of 10 mW/cm² (SAR 2 mW/g) daily for 3 hours from day 4 of pregnancy (in utero) through day 40 post partum. The animals were killed and the brains were removed, weighed, measured and histologically examined at 15, 20, 30 and 40 days of age. The results showed that there was no significant effect on rat brain development due to the exposure during embryonic, fetal and postnatal period. Fertilized Japanese quail eggs were continuously exposed to 2.45 GHz microwave radiation from day 1 through day 12 of incubation to a power density of 5 mW/cm² (SAR = 4.03 mW/g). Irradiated and control embryos were removed from the eggs on day 12, 13 or 14 of incubation, and the cerebella were histologically examined. In order to determine the effects of microwave exposure during embryogenesis on subsequent development of the cerebellum, some of the quail were allowed to hatch and reared to 8 weeks of age. In the irradiated embryos a slight development retardation was found in the cerebellar cortices in terms of several morphological parameters. In the 3 week old quail, no significant differences were noted between irradiated and control cerebella in the morphological measurements of Purkinje cells.

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this project was to determine the effects of 2.45 GHz microwave radiation on brain development. Pregnant Sprague-Dawley rats and fertilized Japanese quail were exposed. Pregnant rats were exposed from above to a power density of 10 mW/cm^2 day for 3 hours from day 4 of pregnancy through day 40 post partum. The animals were killed, and the brain were removed, weighed, measured and histologically examined at 15, 20, 30 and 40 days of age. The histological parameters examined included the cortical architecture of the cerebral cortex, the decline of the germinal layer along the lateral ventricle, the myclination of the corpus callosum, and the decline of the external germinal layer of the cerebellar cortex. Fertilized Japanese quail were also continuously exposed to 2.45 GHz microwaves from day 1 through day 12 of incubation at 5 mW/cm^2 (SAR = 4.03 mW/g). Irradiated and control embryos were removed from eggs on day 12, 13 or 14 of incubation and the cerebella were histologically examined. Some quail were allowed to hatch and were reared to 8 weeks of age in order to determine residual changes. The extend of dendritic abroes, the length of the stem of primary dendrite and the size of the perikaryon of Purkinjc cells were measured in Golgi Cox impregnated sections.

MAJOR FINDINGS AND PROPOSED COURSE: In the exposed Sprague-Dawley male progeny no remarkable differences between microwave exposed and control groups for any of the histological or guantitative parameters were observed. This data indicates that there is no significant effects on rat brain development due to 2.45 GHz microwave exposure during embryonic, fetal and postnatal periods at a incident power density of 10 mW/cm^2. In the irradiated Japanese quail embryos a slight retardation was found in the development of the cerebellar cortices in terms of several morphological parameters. The effects included the growth and subsequent decline of the external granuler layer, the differential growth of the molecular layer, the cellular differentiation and the alignment of Purkinje cells and the accumulation of granule cells beneath the Purkinje cell layer, as well as lower body and brain weights. In the 8 week old quail, no significant differences were noted between irradiated and control cerebella in the morphological measurements of Purkinje cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Reports in the literature has shown that developing embryos when exposed to microwave radiation have behavioral decrements when they reach maturity. This research provides information on the development of the brain which assists in the interpretation of this data as well as provide original research on the effects of microwaves on brain histology.

RESEARCH TRIANGLE INSTITUTE
NO1-ES-9-0008

TITLE: Investigation to Determine the Peripheral and Central Receptor Mediating Effects of Microwave Radiation on Brain Activity

CONTRACTOR'S PROJECT DIRECTOR: Blake S. Wilson, Senior Research Engineer

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: June 1, 1979

CURRENT FUNDING LEVEL: $51,273

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the research are to identify the biological structures that transduce microwave radiation into auditory activity and to investigate possible effects of microwave radiation on the metabolic activity of brain structures outside the auditory system.

METHODS EMPLOYED: Responses of single fibers in the auditory nerve of cats will be recorded using glass micropipette electrodes while the head is being exposed to either continuous wave or pulsed microwave radiation. Post stimulus time histograms of the neural discharge data will provide latency information which can be used to determine the site of interaction. To investigate the interaction of microwaves with various brain centers, patterns of [^{14}C]2-deoxy-D-glucose ([^{14}C]2 DG) uptake in brain centers will be measured. Brain metabolism can then be measured by obtaining autoradiographs of specific loci of the brain.

MAJOR FINDINGS AND PROPOSED COURSE: Autoradiographic maps of brain activity in rats exposed to pulsed or continuous-wave (CW) microwave radiation were made using [^{14}C]2-deoxy-D-glucose. The results showed that both CW and pulse microwaves can elicit auditory responses in the animals. By ablating one middle ear, the measured symmetrical pattern of uptake of [^{14}C]2-DG at the interior colliculus and medial geniculate body showed that the microwave stimulus bypasses the middle ear in eliciting the auditory response.

Single unit recordings in the auditory nerve when exposed to pulse microwaves have been measured in cats and results analyzed. It was discovered that pulses of microwave radiation appear to stimulate fibers in the auditory nerve irrespective of their characteristic frequency. Also, most evidence seemed consistent with the observation that thresholds to microwave pulses are related directly to thresholds in the same units to acoustic clicks. These findings along with confirmation of short-latency responses ($\simeq$ 700 μsec) in the auditory nerve to microwave pulses, are of major importance in that they show the microwave-induced wave of intracranial pressure to be "pulse-like" in frequency content rather than a "sine-like" oscillation as suggested by other researchers. The post-stimulus-time (PST) histograms of some single units showed both short latency ($\simeq$ 700 μsec) and longer latencies ($\simeq$ 1.2 msec) which indicates that the

microwave stimulus is transduced both through the standard auditory pathway and by direct interaction at the hair cells. It is hypothesized that the short latency stimuli are due to field-induced forces at dielectric interfaces within the organ of Corti.

This project will be completed during this fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At the present time the mechanisms of microwave interaction with the brain are unknown. This study provides information which shows that the auditory system is stimulated by both CW and pulse microwaves. It also indicates that the more than one mechanism and site of interaction are involved. This study has broadened our base of understanding of how microwaves interact with peripheral and central receptors of microwave radiation on brain activity.

NEW YORK UNIVERSITY

TITLE: "Photodegradation of Adsorbed Polycyclic Arenes"

PRINCIPAL INVESTIGATOR: Joan M. Daisey, Ph.D., Institute of Environmental Medicine, NYU Medical Center

PROJECT OFFICER (NIEHS): Colin F. Chignell, Ph.D., Chief, Laboratory of Environmental Biophysics

DATE GRANT INITIATED: December, 1978 (Renewed December, 1979)

CURRENT ANNUAL LEVEL: $26,000

GRANT DESCRIPTION

OBJECTIVES: Polycyclic aromatic hydrocarbons (PAH) in the atmosphere can be associated with particle substrates which differ considerably depending upon source. These differences in substrate composition can have a substantial impact upon the PAH half-lives, reaction products and biological activity. As there is little or no information on the half-lives of adsorbed PAH, an investigation has been initiated of the photodegradation of these compounds under simulated environmental conditions. The objectives of this study are:

1. To design, construct and evaluate a fluidized-bed photochemical reactor for laboratory studies of photodegradation of adsorbed PAH;

2. To determine the stability of some PAH epoxides (possible intermediates in photodegradation) adsorbed onto various substrates;

3. To investigate the rates and products of photodegradation of adsorbed PAH on several substrates under various conditions of temperature and humidity.

METHODS EMPLOYED: The photoreactor consists of a glass column 24 cm in diameter. Particles are suspended to a height of about 10 cm by the flow of air or other gases through a fritted disc at the base of the column. An alpha particle emitting source in the reactor eliminates static charging of the particles. The column of moving particles is irradiated by a 200 watt quartz mercury vapor lamp with a reflector which is mounted 15 cm from the center of the column. The glass reactor acts as a filter for ultraviolet wavelengths giving a spectrum in the reactor which is similar to sunlight. Light intensity in the reactor from 297 to 366 nm is about 30 watts/m^2, approximately half the intensity of solar irradiance for this region of the spectrum at 0° zenith angle.

MAJOR FINDINGS AND PROPOSED COURSE: Using the photoreactor, the rates of degradation of pyrene on glass beads and on Carbosieve S have been determined. In general the rates are reproducible to within ± 20-30% from one experiment to the next. When the reaction is followed over the same time interval, rates appear to be reproducible to within 20%.

The rate of degradation was found to follow a first order kinetic rate law but the half-life of pyrene was strongly dependent upon the substrate. The half-life of pyrene in the presence of air and light was 1.0 ± 0.2 hrs. on glass beads and 31 ± 6 hrs. on Carbosieve S, a pure carbon substrate.

Within the experimental uncertainties of the determinations, the rate of degradation of pyrene in the presence of light did not differ from that in the absence of light. In the case of the glass beads, in an experiment in which nitrogen was substituted for air, there was no detectable loss of pyrene over a period of several hours. Thus, oxygen appears to be required for the degradation reaction although light is not.

Extracts of samples of pyrene on glass beads and on Carbosieve S which had been exposed to air and light in the photoreactor were analyzed by HPLC. In the case of the glass substrate the chromatograms indicated the formation of 7 products. Although standard pyrene derivatives were not available at this time, the chromatograms suggest that quinones and bipyrene may have been formed.

For the Carbosieve S, there was no evidence of the presence of quinones in the extracts. Several pyrene quinone derivatives as well as 1,1-bipyrene were available at the time of these experiments. It is not clear at present whether these compounds are not recovered from the carbon substrate or do not form. The formation of bipyrene was observed both with and without light. Compounds eluting at the same retention times as fluoranthene and benzo(e)pyrene were also observed, suggesting the possibility of rearrangement reactions of pyrene on the surface of carbon. Experiments to confirm the identities of these compounds are in progress.

These first experiments on the degradation of pyrene under simulated environmental conditions have shown that the photoreactor which has been developed can provide potentially useful information on the rates and products of degradation of PAH and the factors which influence them. Further experiments with other PAH's, under a variety of conditions, can provide needed information for the assessment of environmental and occupational health hazards of airborne PAH in the context of changing fuel use patterns.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Effluents from such processes as oil shale conversion and coal gasification contain high concentrations of PAH. Once released into the environment these agents may be adsorbed onto soil particles and other substrates. Since photochemical degradation in the presence of oxygen could give rise to intermediates that are more chemically reactive than the parent compounds it is of importance to isolate and identify such compounds.

PUBLICATIONS

Daisey, J.M. Organic compounds in urban aerosols. Ann. N.Y. Acad. Sci. 338: 50-69, 1980.

Daisey, J.M., R.J. McCaffrey, and R.A. Gallagher. Polycyclic Aronatic Hydrocarbons and Total Extractable Particulate Organic Matter in the Artic Aresol. Atmospheric Environment. 1981.

LABORATORY OF ENVIRONMENTAL CHEMISTRY

LABORATORY OF ENVIRONMENTAL CHEMISTRY
Summary Statement

The goal is to utilize inter- and intradisciplinary strategies in chemistry and biology to understand the causes of environmentally related human health diseases at the molecular level so that preventive or control measures may eventually evolve. This conceptual approach has been termed "environmental health chemistry" and as we practice it, deals with the molecular level information needed to assess potential human health hazards associated with chemical contamination of our environment. In environmental chemistry studies, the laboratory stresses the importance of providing definition of research chemicals for biological study and emphasizes the need to study chemicals as compound classes where possible. In this regard, the laboratory has focused particular attention on the persistent pollutants especially those that tend to bioaccumulate.

In addition to continually developing new and improved methods in analytical and synthetic chemistry, the laboratory emphasizes mechanism elucidation at various biochemical and molecular levels including development of structure-activity correlations as a predictive tool in toxicology. There are three work groups which form the basis for these research activities within the Laboratory, viz. Analytical Chemistry, Biochemistry and Organic Chemistry. Each of these groups has certain distinguishing characteristics with respect to expertise and instrumentation which make it a unique resource to the Institute.

RESEARCH ACTIVITIES

Analytical Chemistry

The analytical chemistry program has available in-house one high resolution mass spectrometer/mass analyzed ion kinetic energy spectrometer, one additional high resolution mass spectrometer for support activities, a tandem quadrupole system for mass spectrometry/mass spectrometry and a quadrupole instrument for positive or negative ion chemical ionization studies which has been interfaced to a high pressure liquid chromatograph. All mass spectrometers except the chemical ionization quadrupole system are interfaced to gas chromatographs. Two modern computer data systems are available so that any three instruments can be interfaced at a given time. The group also maintains an analytical gas chromatograph with flame ionization and electron capture detectors and a high pressure liquid chromatograph for program use. It is planned to add an additional data system as part of the facility. The program has considerable research activity beyond the boundaries of the Institute in various collaborative research programs. The in-house research activity can be conveniently divided into four areas: gas phase ion chemistry, negative chemical ionization mass spectrometry, analytical methods development and applications of analytical mass spectrometry.

Mass spectrometry continues to be one of the most powerful techniques available for trace organic analysis. After thirty years of studying the electron impact induced reactions of organic molecules, a skilled mass spectrometrist can frequently identify an unknown compound on the basis of its mass spectrum. Newer variations of mass spectrometry do not have the advantages of this great body of knowledge. The rapidity with which positive ion chemical ionization mass

spectrometry spread through analytical mass spectrometry can be attributed to the basic understanding of positive ion-molecule reactions developed beginning in the late 1950s. However, the major reaction utilized at present is simple proton transfer. The development of negative ion chemical ionization as an analytical method is being impeded by a shortage of basic knowledge of negative ion-molecule reactions. The fragmentation reactions observed in the collisional activation process have received very little attention. Since these are fundamental to use of tandem mass spectrometry for analytical purposes, it is of critical importance to better understand them. By performing mass analysis then energy analysis on an ion beam, it is possible to measure the amount of internal energy of the fragmenting ion. Since the ions are relatively old (lifetime ca. 10^{-5} s) by the time the ions of interest fragment, they have little energy more than necessary for the reaction to occur. This should be a fundamental property of that ion. Presuming the ion still contains information related to the structure of the molecule, this kinetic energy release should be useful for structural analysis.

The collision activation process is useful for inducing fragmentation reactions for otherwise stable ion beams. Chemical ionization (CI) using H^+ transfer is widely used to confirm molecular ion assignments. This is based upon the $(M + H)^+$ ion being the major ion of the CI mass spectrum. While the molecular weight is an extremely useful piece of information about an unknown molecule, the structural features revealed by fragmentation reactions is valuable data. In order to see if it is possible to benefit from both and add to our knowledge of collision-induced fragmentation reactions we obtained the collision induced dissociation-mass analyzed ion kinetic energy (CID-MIKE) spectra of a number of compounds.

The charge reversal experiment is interesting for a number of reasons, not the least of which is its novelty. We studied the charge reversal of a number of relatively simple negative ions in order to gain some understanding of the process itself, to see if useful information concerning negative ions could be obtained and to prepare and study unusual positive ions.

One of the basic questions of the collisional activation process concerns the amount of energy available to induce fragmentations. By studying the CID-MIKE spectra of carbon cage compounds cubane, kepone and mirex we found that sufficient energy is available for cage rupture; i.e., at least 9eV. Also sufficient time is available between energy acquisition and subsequent unimolecular fragmentation for the energy to become concentrated in three bonds to the same carbon atom. The CID-MIKE spectra of $C_5Cl_6^+$ from mirex and hexachlorocyclopentadiene are virtually identical. Likewise, the $C_6H_6^+$ ions from cubane and benzene give similar spectra.

As alluded to earlier, the kinetic energy release associated with a fragmentation reaction can be useful for ion structural analysis. If one presumes the structure of the molecular ion is related to the structure of the original molecule, then kinetic energy release measurements have the potential of being useful for unknown identification. Therefore, unimolecular and collision induced decompositions of the major ions of selected polychlorinated biphenyls (PCBs) were studied. Loss of a single chlorine atom is associated with a wide range of kinetic energy releases but still can be correlated by a single reaction mechanism. Loss of two chlorines is interpreted as a rapid sequential loss from isomerized molecular ions for all but one compound. The decompositions which metastable ions undergo

are not always the same as those of higher energy ions in the source. Correlations between substituent positions and kinetic energy release can be made for the $M^{+\cdot} \rightarrow (M\text{-}Cl)^{+} \cdot$ and $(M\text{-}Cl_2)^{+} \cdot$ processes.

We shall continue to study the kinetic energy release associated with the fragmentation of selected environmental compounds to help in establishing their "chemical appearances". We are studying the charge reversal process by comparing the charge-reversal spectrum of negative ions with the CID-MIKE spectra of positive ions which can be assumed to have the same structure. An instrument is under construction in collaboration with Professor Thomas Baer at UNC-Chapel Hill which will permit the study of gas phase isomerization reaction.

Negative chemical ionization (NCI) mass spectrometry has been found useful for selected environmental analytical problems. At present, our basic knowledge of the behavior of environmentally interesting chemicals under various NCI conditions is one of the major limiting factors on the application of NCI to specific problems. Previous work in our laboratory has shown NCI to be useful for the determination of polychlorinated dibenzo-p-dioxins (PCDD) in bovine tissue. A concurrent investigation of the mass spectrometry of the available PCDDs indicated that O_2 as a reagent gas showed great promise as a highly specific means of detecting 2,3,7,8-TCDD. Unfortunately, we have never had a sufficient number of reference standards to demonstrate this to our satisfaction. Also we were interested in the NCI behavior of related compounds so that we could assess their potential to interfere with PCDD determinations. Thus we investigated the NCI behavior of polychlorodiphenyl ethers (PCDE) and 2-hydroxypolychlorodiphenyl-ethers (predioxins).

The negative ion spectra of selected organic nitriles were measured using methane NCI. These spectra contained abundant $(M\text{-}1)^{-}$ ions and few fragments. Ion molecule reactions with residual oxygen, methane and between sample molecules and ions form diverse products with low abundances relative to the $(M\text{-}1)^{-}$ ions. Detection limits generally are comparable to those for positive ion methods, and for selected compounds, can be several orders of magnitude lower. These results suggest that negative ion methods can be useful for molecular weight measurement *via* the $(M\text{-}1)^{-}$ ion.

We were able to confirm the existence of the monomeric metaphosphate anion (PO_3^{-}). This is the first direct observation of a widely postulated intermediate in phosphorous chemistry.

The NCI program at NIEHS has evolved to the point of being available in the research support program. Thus a large part of future research will be addressed to specific problems which arise in support of other research programs. The NCI mass spectrometer has been modified to permit interfacing a high pressure liquid chromatograph. This facility will provide positive or negative CI and utilizes an interface similar to that proposed by Baldwin and McLafferty.

In general, advances in biomedical sciences have followed the development of analytical methods which permit the study of problems which were previously inaccessible. For example, the impact of combined gas chromatography/mass spectrometry (GC/MS) on the biological and environmental sciences has been immense and is still increasing as more researchers gain access to instruments which themselves are becoming even more powerful and versatile. Even with the full capabilities of modern GC/MS instruments, a large fraction of the questions

facing biomedical researchers are still beyond our abilities to perform the necessary assays. There are a number of reasons for this. First of all, the sample must have suitable volatility and thermal stability to withstand the rigors of gas chromatography. This eliminates more than 80% of the organic compounds known to man and most chemicals of biologic origin. Second, GC/MS may not offer sufficient sensitivity for a particular assay. Third, GC/MS may not offer sufficient specificity for an assay.

We have designed and implemented modifications to one of our high resolution GC/MS/Computer systems which permits the acquisition and storage of individual mass spectral peak profiles in a selected-ion-monitoring without detectable loss of chromatographic resolution with a high resolution capillary column. Although generally useful, we have applied this technique only to the TCDD problem thus far.

It is recommended practice in analytical chemistry to confirm a result by an independent method before reporting quantitative results. For the particular problem of TCDD determinations, one must rely on GC/MS for its own confirmation. One obvious choice is re-analysis on a GC column of different selectivity. We feel it is also desirable to alter the mass spectrometry as much as possible. Thus we have evaluated the use of CID-MIKES spectrometry in the single reaction monitoring mode as a GC detector for TCDD confirmation. Signal:noise of 5:1 for the transition $M^+ \rightarrow (M\text{-}COCl)^+$ could be obtained for 2-5 x 10^{-12} g. Thus, the confirmation detection limit is ca. an order of magnitude greater than that of the exact mass measurement detection limit. By combining the two techniques, we require that an unknown have the same exact mass as TCDD ± 10 ppm, the same chromatographic retention time as TCDD by co-injection and the same quantitative response by EI and CID-MIKES before we report a positive result. We require no response (S:N<3) before we report a negative result. The intermediate cases are reported as negative at the higher detection limit and unconfirmed positives at the level indicated by the more sensitive technique.

The use of CID-MIKE spectrometry and, more recently, tandem mass spectrometry utilizing two (or more) quadrupole mass filters (MS/MS) is being proposed for the analysis of crude mixtures by direct probe sample introduction. This method is obviously limited if isomeric materials are present. In addition, we have investigated what limitations the matrix might impose from non-specific interferences. We found a practical dynamic range of ca. 10^4:1 for matrices known to be free of specific interference.

We have acquired the necessary parts and constructed a tandem quadrupole MS/MS instrument using a collision cell made of a leaky dielectric which allows penetration of rf fields only. Using this instrument, we have compared the resulting low collision energy dissociation products with the high collision energy products from the MIKE spectrometer. It is not surprising that the high energy spectrum contained more fragments than the low energy one. Nor was it surprising that the high energy fragments were not easily related to neutral structure. Thus, while the high energy spectrum gives a more detailed fingerprint, those spectra contained little if any information not available in the low energy spectra. Furthermore, the extra fragment peaks increase the likelihood of interference in the case of an impure sample.

Field desorption (FD) mass spectrometry has shown some promise as a tool for the analysis of relatively pure samples of limited volatility. Therefore, we have

developed an in-house FD capability and are studying a number of compounds of interest to the NIEHS.

The use of CID-MIKES spectrometry for qualitative and quantitative analysis will be pursued. Techniques for compound class specific analysis by constant neutral loss scans of the MS/MS instrument will be explored. The use of kinetic energy release as a structural probe will be studied further.

The solution of many environmental health problems is frustrated by the lack of appropriate chemical methodology. The problem addressed by other work is to provide state-of-the-art GC/MS instrumentation and highly competent mass spectrometrists to participate in collaborative research projects. In the past 30 months, the support capabilities have expanded from only low resolution GC/low resolution MS to high resolution GC/high resolution MS with exact mass measurements, positive and/or negative chemical ionization GC/MS, high resolution selected ion monitoring and metastable ion analysis being available to any intramural scientist who can demonstrate a legitimate need. Furthermore, no collaborative project has been rejected on any grounds other than non-feasibility.

Biochemistry

Biochemistry is the study of living things at the molecular level. The all-emcompassing definition accurately reflects the immense range of investigations that can be termed biochemistry. In general, the biochemistry program serves three major functions in the Laboratory. It serves as a communication interface between the analytical and organic chemists and the biology-oriented laboratories of the Institute, it develops methods for coping with the need to analyze for trace levels of chemicals in biological matrices, and it does research on the mechanisms of action of environmental toxins at the molecular level with emphasis on metabolic factors and the immune system.

Our communications role involves close interaction (including collaborative research) both with the other chemists in the Laboratory of Environmental Chemistry and with investigators in other laboratories both inside and outside the Institute. We influence the thinking of both groups in regard to the environmental relevance of their work.

Much of our work in bioanalytical methods development concerns the difficulties in converting an extremely complex "extract" from tissues or other "dirty" matrices into something to which analytical chemists can apply their determinative techniques. In addition we develop true "bioanalytical" techniques (such as radioimmunoassays) as alternatives to conventional analytical methods and as independent means of confirming the results of other methods.

Our biochemical research deals primarily with two general questions: what role does metabolism play in the mechanisms of action of toxins, and in what manner is the toxic mechanism manifested at the molecular level? We could undoubtedly attack these questions much more effectively if this aspect of our program were able to grow in personnel.

Several environmental agents have thus far resisted all efforts to elucidate their mechanism(s) of toxicity at the molecular level. In some cases this may reflect the fact that they interact with biochemical systems whose normal functioning is not adequately understood. In other cases toxicity may be

dependent upon only one of several concurrent routes of metabolism, the relevant intermediates not having as yet been identified. Toxicity may be indirect and far removed from events immediately involving the test compound. In such cases as these, elucidation of the underlying mechanism(s) of toxicity requires input from a range of biochemical investigations, including studies of uptake, transport, and distribution, intermediary metabolism, homeostasis, structure-activity modeling, and continually increasing comprehension of the details of normal functioning of biological systems. Examples of compounds whose mechanisms of toxicity are unknown in spite of extensive investigation include: 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds (which can cause organ cyto-necrosis at burdens of less than one molecule per cell in sensitive species), polychlorinated biphenyls (which may operate by a mechanism analogous to that of the dioxins or may not), polynuclear aromatic hydrocarbons, whose carcinogenic potential is beginning to make sense relative to specific metabolites, and di-(2-ethylhexyl) phthalate, which causes male infertility at moderate doses and reportedly, liver cancer at high doses in spite of no evidence for tissue accumulation.

At the present time we have only modest objectives in this area due to the small number of investigators available in the Laboratory of Environmental Chemistry. In the aspect of basic research into biochemical functions, we are attempting to relate strain and species variations in susceptability to toxins to variations in isoenzyme distributions for key enzymes. We are beginning to concern ourselves with the normal role of cytochrome P-448-dependent oxidations in homeostasis. We wish to determine the role, if any, of the cytoplasmic "receptor" for TCDD in its toxic manifestation. We are investigating the ability of specific portions of the DEHP molecule to become covalently bound to DNA as a possible explanation for its reported carcinogenic potential.

Presently, used assays for the "cytoplasmic receptor" that binds TCDD are either extremely inaccurate (requiring correction for 98 + % nonspecific binding) or extremely tedious (requiring sucrose gradient centrifugation or isoelectric focusing). Making use of the principles developed for radioimmunoassay of TCDD, we have devised a binding assay for the receptor that has relatively little nonspecific binding and therefore requires no extremely elaborate correction procedures. We have begun to use this assay in the purification of the receptor.

Metabolic events caused by TCDD have been detected within hours after dosing in exploratory studies. In general, these events represent the initiation of possible self-perpetuating reactions, possibly involving free radicals. Qualitative differences in the early response of rats to sublethal and lethal doses have been seen in various aspects of lipid metabolism.

The qualitative ability of various PCB isomers to serve as inducers of cytochrome P-450-dependent, cytochrome P-448-dependent, both types or neither type of mixed function oxidase activity has been correlated with the molar polarizability of the PCB isomers in a manner permitting the prediction of activity of untested isomers. The freedom from a requirement for metabolism has been shown. Since PCBs may be the simplest molecular structures showing these few types of activity, this approach provides the potential of visualizing the initial step in the induction process.

Molecular polarization studies will be applied to carcinogenic and noncarcinogenic polynuclear aromatic hydrocarbons to assess the generality of this predictive

approach. The relationship between TCDD toxicity and its ability to (a) induce P-448 and (b) initiate free-radical processes in membranes will be further investigated through the use of specific inhibitors. Affinity chromatography using receptor-binding analogs of TCDD coupled to a solid support will be investigated as a means of isolating the receptor. The metabolism of DEHP is presently being studied in mice.

Carcinogenicity at high doses of diethylhexyl phthalate ester may be related to observations that, while the phthalate moiety does not bind to macromolecules, and 2-ethylhexanol does not bind to DNA, the 2-ethylhexyl moiety of DEHP is covalently bound to DNA when high doses are given. There are at least two "threshold" effects in the dose-response curve. Other evidence, especially metabolic studies, strongly suggest that rats and mice may be very poor models for man in the handling of DEHP. The African Green Monkey, in contrast, may be a suitable analogue.

It is often more difficult to judge the validity of an analytical study than to perform the analyses. Perhaps the best general description of the problem was given by J.S. Hunter in "The National System of Scientific Measurement", Science, 210:869 (1980). His summary states, "Mandated measurement methods are required by regulatory agencies and other government groups. . . Few provide adequate estimates of precision, and fewer still provide any evaluation of interlaboratory bias. The societal costs of these poor measurements are large. Much needs to be done to meet the physical and statistical requirements for establishing and maintaining dependable measurements."

In the context of environmental analysis, we have become aware that methods for the determination of numerous compounds of interest are insufficiently sensitive, inadequately specific, occasionally unreproducable, and inevitably lacking in validation. While in most cases the final measurement step is least inadequate in these areas, the preliminary sample handling, extraction, and cleanup procedures usually degrade the overall quality of the method. Principles governing the data that must be generated to constitute validation of sample workup procedures have been lacking, and the result has been inadequate, haphazard validation efforts in many cases.

The types of environmental analysis problems we have been concerned with recently can be exemplified by three different objectives. A need for the reproducible determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at a level of 1 part-per-trillion in tissues has existed for several years. Problems to be overcome include interference from other TCDD isomers, disadvantages of current internal standards, inadequate criteria of identification, unreproducible extraction, and cleanup recoveries due to handling losses, and interference from other halogenated aromatics. In the case of the polychlorinated biphenyls, a need exists for a method to determine "total PCB content" that is not invalidated by metabolic or environmental alteration of the PCB mixture, and also a method to determine levels of each individual isomer present. None of the methods in current use meet these requirements. The third example is a type of "general unknown" problem. Many types of environmental samples contain not only mixtures of compounds (e.g., PCBs), but mixtures of classes of compounds. Used transformer fluid, "Yusho" rice oil, and human adipose tissue are examples we have encountered. Means to determine the amounts of each class at the PPB or PPM level have been lacking.

For the TCDD problem we are testing the ability of various compounds to serve as non-interfering "carriers" to prevent or minimize handling losses. We are systematically screening compounds as candidate internal standards. Such a standard must not be discriminated from TCDD during extraction or cleanup, but must be easily distinguished during the measurement step. It must not contain TCDD as an impurity (as most current internal standards do). We are attempting to develop two-dimensional capillary column gas chromatography to minimize interferences. We approach the PCB problem in two ways: one approach is radioisotope dilution-perchlorination. The principle has been shown quite practical, but impurities in available reagents limits sensitivity. The second approach is to develop a gas chromatographic technique that can separate all 210 PCB compounds in a reasonable length of time, along with a means of internal standardization. The "general unknown" problem is approached through a preliminary class fractionation scheme, selected ion monitoring mass spectrometry, and conversion of particular classes of compounds to single compounds either by perchlorination or dechlorination as appropriate.

We have demonstrated the ability of a non-interfering carrier (trichlorodibenzo-p-dioxin) to nearly eliminate handling losses in the analysis of TCDD and have identified several factors responsible for such losses. We have synthesized several compounds as potential internal standards, but have yet to select the optimum one. Two-dimensional GC has been shown to work in principle, but has not yet been optimized for capillary columns. The ineffectiveness of several commonly used cleanup steps has been demonstrated. A reliable method for determination of "Total PCB content" has been developed, but needs improvement to reach below the 1 ppm level. Means to separate all 210 PCBs has been developed and applied to the complete characterization of Aroclors 1221, 1242, 1016, 1248, 1254, and 1260. A method for class analysis of complex mixtures of halogenated aromatics has been developed and applied to the analysis of used transformer fluid. For the first time polychlorinated terphenyls have been detected and measured in Yusho rice oil. Several papers and talks on validation of workup procedures have been given or are in preparation.

Besides these more specific objectives, we are also concerned with developing principles for the validation of proposed work-up procedures, particularly in the areas of sample spiking, extraction, and cleanup. Validation principles are derived largely through common sense and experience, and by discussions with representatives of NIOSH, EPA, FDA, etc. Having these principles actually accepted and applied, however, is to a considerable extent a selling job approached by publications and presentations giving examples of real benefits derived from such activities.

Complete formulation of an analytical scheme for the 1 ppt determination of TCDD is awaiting availability of synthesized standards of other TCDD isomers to evaluate interferences. These syntheses are to be dealt with through outside contracts. Our present GC method for PCBs requires 5-1/2 hours. We are investigating means to shorten the time by computer processing of output data. We are standardizing procedures for measuring the radiochemical purity of commercial products, since we commonly find the quality control methods of the manufacturers inadequate. We are now moving from the "developing methods for the validation of methods" stage to the actual validation of methods, especially in the areas of extraction and cleanup.

Immunochemists have tried many times in the past to take advantage of the very high sensitivity and specificity of immunoassay techniques by applying them to the determination of pesticides and other compounds of low water solubility, generally without success. [For a review see Ercegovich, C.D., "Analysis of Pesticide Residues: Immunological Techniques", in Pesticides Identification at the Residue Level, Advances in Chemistry Series 104, A.C.S. (1971)]. If such techniques were available, they would provide an independent means of confirmation of gas chromatographic or GC-MS data, as well as making low-cost residue analysis possible to clinical laboratories. In many cases, unfortunately, the compounds against which one would like to raise antibodies are themselves immunosuppressive, and lack reactive sites at which coupling to an immunogen could occur, in addition to their insolubility in the aqueous media needed for antibody-antigen binding to occur.

We have succeeded in overcoming most of the problems associated with immunoassay of halogenated aromatic environmental chemicals. Thus far, effective radioimmunoassays for chlorinated dibenzo-p-dioxins (detection limit 20 pg), chlorinated dibenzofurans (detection limit 20 pg), and polychlorinated biphenyls (PCBs) have been developed. The PCB application is a nanogram level "fingerprinting" technique that permits the identification of, for example, a particular Aroclor in the presence of excess chlorinated naphthalenes or chlorodiphenyl ethers that obscure the GC pattern. We have also developed a radioimmunoassay for mono-2-ethylhexyl phthalate, a contaminant of blood stored in PVC blood bags. The radioimmunoassay for dioxins has been patented. A special feature of these assays is a general technique for solubilizing hydrophobic compounds in a manner permitting their binding to antibodies.

Radioimmunoassays for p,p-DDE and brominated naphthalenes are under development. Unexpected difficulties have arisen in regard to developing a radioimmunoassay for 2,4,5-trichlorophenoxy acetic acid. Understanding the reasons for the difficulties may increase our general understanding of competitive binding (protein-ligand), so studies involving this hapten will continue. We would like to institute hybridoma studies in order to generate more specific antisera both for assay purposes and to enable us to more meaningfully study the structural aspects of antibody-hapten binding, but we lack sufficient personnel, to assign to this project. Due to personnel and budgeting constraints, we have been unable to replace those members of the immunochemistry unit lost by attrition. We are also interested in investigating solid-phase immunoassays, which should greatly simplify sample preparation.

The second aspect of our immunobiochemistry studies is the investigation of the mechanisms of immunosuppression or immunostimulation by immunoactive environmental agents. Parameters investigated include target cell types, dose and time dependence, degree of alteration, reversability, age and sex dependence, effects direct or dependent on metabolism, etc. A significant objective is the establishment of a battery of bioparameter measurements as reliable screening tools for both detection and elucidation of undesirable immunoactivity. This aspect of our research is heavily collaborative in nature.

Many compounds of interest to environmentalists interact with the immunologic systems of animals. In some cases the interactions lead to harmful reductions in the immune competence of the animals, i.e., decreased ability to resist infections with bacteria and/or viruses, decreased ability to cope with spontaneous tumor cell appearances, etc. In the most conspicuous immunotoxic

responses, specific tissues or cell types associated with the maintenance of immunocompetence, e.g. thymus, bone marrow, etc., serve as target tissues for damage or destruction by the toxins. In other cases the toxic effects are more subtle. At the present time it is impossible to predict a priori whether a given chemical (not previously tested) will interact with the immune system and, if so, with what result.

The effects of pre/postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on various immunological, bone marrow and host susceptibility assays were examined previously in $B_6C_3F_1$ hybrid mice. These studies were continued with several other compounds including a biphenyl (non-halogenated) chemical, orthophenylphenol. The effects of orthophenylphenol (OPP) and tris(2,3-dichloropropyl)phosphate (Fyrol FR2) on immunological functions and host susceptibility to infectious agents were examined following subchronic exposure to nontoxic levels in adult mice. Also included in these studies as a positive control were mice treated with cyclophosphamide (CY), a known immunosuppressant and cytoreductive drug. Exposure to relatively high doses of OPP (up to 200 mg/kg body weight per day for 10 days) failed to alter immune functions or host susceptibility. Fyrol FR2 treatment induced minimal changes in immune functions and host susceptibility only at the highest dose tested (25 mg/kg per day for 4 days) as indicated by decreased lymphoproliferative responses to mitogens and increased tumor takes following tumor cell challenge. In contrast, CY treatment resulted in marked alterations in both immunological functions and host susceptibility.

The relationship of various carcinogens and structurally similar noncarcinogens are being examined in relationship to immune alteration. We have examined benzo(a)pyrene and benzo(e)pyrene as well and methyl and ethyl carbamate pairs. These studies have shown that B-cell but not T-cell function are impaired by the carcinogens but not the noncarcinogens. Urethane (ethyl carbamate) appears to have a preferential affect on the bone marrow. Myelotoxicity can be demonstrated far below levels which induce general toxicity or immunotoxicity.

The DES induced immunosuppression has been described in the previous annual report. We are presently examining the mechanisms of DES-induced immunosuppression at the cellular and molecular levels by examining suppressor substance produced by macrophages of DES treated animals and various changes in macrophage enzymes and alterations in metabolic pathways.

The effects of immune elimination of helminthic parasites from mice exposed to various chemicals of environmental concern are being studies. Evaluation of adult worm longevity in exposed animals indicated that selected chemicals adversely affect the ability of treated hosts to respond normally to a helminthic infection. The T. spiralis model offers an extremely sensitive indicator for determining altered host susceptibility following chemical exposure and may play a role in increased human parasitic infection in persons exposed inadvertently to selected chemicals.

Immunotoxicology studies are rapidly coming under the auspices of the National Toxicology Program and are expected to be essentially deleted from the Laboratory of Environmental Chemistry internal program by the time we move into the permanent facility. Since many of the techniques used in these studies were developed by Laboratory of Environmental Chemistry personnel, we retain interest in this research and will presumably remain available for collaboration.

Organic Chemistry

The majority of research in the Organic Chemistry Group for this reporting period involves some aspect of the detoxication of polycyclic aromatic hydrocarbons (PAHs) by the glutathione S-transferase enzymes. Research is conducted in three broad categories: (1) synthesis of PAHs and their oxygenated metabolites; (2) mechanistic investigations of the conjugation of glutathione (GSH) with arene oxides catalyzed by the glutathione S-transferases; and (3) biochemical characterization of purified glutathione S-transferases.

The mechanism by which polynuclear aromatic hydrocarbons (PAH) exhibit carcinogenic activity is now known to involve oxidized metabolites of the hydrocarbons. Although evidence pointed strongly to those metabolites where oxidation had occurred in the "bay region" as being the derivatives responsible for the potent activity of their parent, recent work indicates that some of the many other metabolic products may be involved. It has also been found that there are PAH which do not possess bay regions as part of their structure but which are still mutagenic and carcinogenic agents. Furthermore, some very recent work has investigated the activity of some highly reduced aromatic hydrocarbons which although still "polynuclear" calls into question the generally assumed structural requirements necessary for biological activity. In the light of these new demands on existing theories and rationalizations, new structural variations and derivatives of old established PAH are needed. To prepare these materials new synthetic methodology and strategies will have to be developed.

In contrast to existing methodology, which leans heavily on classical chemical reactions noted for their severe conditions and propensity to yield mixtures of products, we have chosen to explore the Diels-Alder cycloaddition process as a synthetic strategem for the synthesis of PAH. By choosing appropriate starting materials it is possible to prepare, in one or two steps, parent PAH as well as intermediates with the basic PAH framework. These intermediates can also possess functionality appropriate for elaboration to additional PAH. These cycloadditions can be constructed so as to proceed in intra and intermolecular modes. Each of these possibilities lead to different PAH skeletons in a short and direct manner. The products of these reactions are created in a partially reduced form compared to the parent PAH. This feature enables one to introduce functionality at specific sites as desired.

We have been able to realize the basic objectives outlined above. The reactions of benzo(c)thiophene dioxide, a readily available precursor to the transient intermediate o-quinodimethane, and dihydronaphthalene leads to hexahydrobenzanthracene in high yield. In a second step these compounds can be easily oxidized to benzanthracene. If substituted dihydronaphthalenes are used, then substituted benzanthracene are obtained. Alkylation of the starting material, benzothiophene dioxide, and it's use made possible the synthesis of 7 and 12 substituted benzanthracenes. If cinnamic acid derivatives are used in place of dihydronaphthalenes as dieneophiles then intermediates are readily produced which can be elaborated to benzanthracenes substituted at the 5 and 6 positions. In fact even more highly substituted PAH can be attained via this scheme since substituted cinnamic acids allows for synthesis of multiply substituted benzanthracenes. By employing this concept we have prepared benzanthracene and some reduced derivatives specifically labeled with carbon-13 at the 5 position (the K-region).

We have been able to prepare chrysene derivatives by an intramolecular variation of the above process. These reduced derivatives can be selectively oxidized in stages to progressively more highly oxidized PAH, finally leading to the parent chrysene itself.

The ready availability of certain benzanthracene intermediates made it possible to synthesize the previously unknown 4,5-cyclopentenobenzanthracene. We anticipate that this compound will be of some value in testing certain hypothesis concerning structural requirements necessary for activity in PAH.

Preliminary experiments with functionalized substituted o-quinodimethanes have produced benzanthracenes with the potential to be elaborated into benzpyrenes with functionality at the difficulty accessible 11 and 12 positions. Some other preliminary work has promised access to potentially interesting compounds in the 7,12-dimethylbenzanthracene and cholanthrene series. The method can be extended to provide new routes to heterocyclic PAH and their metabolites.

Biologically relevant molecules continue to be synthesized by new or improved methods. The synthesis of optically pure thromboxane intermediates from carbohydrate precursors, and the synthesis of glutathione conjugates of alkene and arene oxides illustrate two of such cases. The first case is a polyfunctional molecule in which groups of similar chemical reactivity have to be selectively transformed to produce the desired intermediate(s). The synthetic difficulties associated with the use of carbohydrates as starting materials are compensated by the benefits derived from using optically pure precursors. The second case exemplifies a situation in which conventional methods are not directly applicable. Although glutathione conjugates, and related thioethers such as mercapturic acids, have been known for many years to result as the in vivo and in vitro alkylation products of epoxide metabolites, a rational procedure for their preparation is not available. Reports in the literature deal mostly with studies on reactions of epoxides with model thiol nucleophiles. A major problem to overcome is the contrasting polarity of nucleophile, highly polar, and electrophile, low polarity.

A synthetic sequence from carbohydrate precursors was completed which provided access to optically pure thromboxane intermediates. A key step in this synthesis involved the selective protection of the hydroxyl group at the C-6 position in glucose as a triphenylmethyl ether. The procedures published for the preparation of triphenylmethyl and t-butyldimethylsilyl ethers have had extremely good acceptance among synthetic chemists. They are well established methods, and may in fact become the preferred procedures for the protection of hydroxyl groups as the above mentioned ethers.

The synthesis of thioether conjugates, N-acetylcysteine or glutathione, of alkene and arene oxides illustrates a systematic approach to the preparation of such metabolites. The phase-transfer method provides a simple way of obtaining mercapturic acid derivatives of epoxides. The compounds, as obtained by this procedure, are soluble in organic solvents and the usual spectral determination are simplified. The synthesis of glutathione conjugates of arene oxides has provided access, for the first time, to non-enzymatic samples of these compounds. Thioethers from other reactive metabolites may be obtained under similar conditions as the ones described for alkene and arene oxides. The synthesis of glutathione and related thioether conjugates will be expanded to include reactions

with other electrophiles such as α,β-unsaturated carbonyls, nitrohaloaromatics, and others.

It is now widely accepted that the metabolism of chemicals in the body can lead to the formation of both more (metabolic activation) and less toxic compounds (detoxication). The cytochrome P-450 dependent monooxygenase system, localized in the microsomal fraction of liver and many extrahepatic tissues, is responsible for the biotransformation of many hydrocarbons to their corresponding arene oxides which have been implicated as causative agents in mutagenesis, carcinogenesis and tissue necrosis. Their toxic effects are thought to result from the covalent binding of the arene oxides with cellular constituents such as nucleic acids and proteins. The two major biotransformation pathways for epoxides are conjugation with glutathione (GSH) which is catalyzed by the cytoplasmic or microsomal glutathione transferases and hydration to the diol catalyzed by microsomal epoxide hydrolase.

The reaction of GSH with electrophilic chemicals constitutes an important part of the detoxication mechanisms available to many species. Although there has been considerable effort devoted to investigating the metabolism and excretion of mercapturic acid (N-acetylcysteine) conjugates of epoxides, there has been surprisingly little work on the structure of the GSH conjugates derived from alkene and arene oxides.

Several questions remained concerning this important detoxication route. What are the structures of the GSH conjugates? Is there a stereochemical preference for the formation of one isomer over another? How do the structures of the conjugates derived chemically compare with those derived from enzymatic conjugation? Is there a stereochemical preference in the further metabolism of the GSH conjugates to the mercapturic acid conjugates? What is the mechanistic role of the transferases in the conjugation reaction?

The chemical conjugation of GSH with styrene oxide produces both possible positional isomers and as a mixture of diastereoisomers (a total of four isomers). The ratio of the isomers is approx. 60:40. Experiments with the optically pure isomers of styrene oxide have allowed the determination of the relative stereochemistry of the four isomers. Similar experiments have established the relative stereochemistry of the mercapturic acid conjugates of styrene oxide. In contrast to the chemical conjugation, the conjugation of GSH with styrene oxide catalyzed by the glutathione transferases (rat liver cytosol) produces the positional isomers and with a preference for the benzylic thioether conjugates of 90%. The results with the styrene oxides clearly show that in making predictions about the possible GSH conjugates produced from oxides, both carbonium ion stability and steric factors must be taken into account.

Preliminary experiments on the enzymatic conjugation of the optically pure isomers of styrene oxide with GSH suggests that there is a significant difference in the kinetics between the two enantiomers. This observation is under further investigation at present.

The metabolism of the glutathione conjugates of styrene oxide by the rat and winter flounder (fish species) has been investigated. Whereas the mercapturic acid conjugates are the major metabolic products in the rat, the major products from the winter flounder are the cysteine conjugates.

^{13}C NMR analysis of the glutathione conjugates formed from ^{13}C-enriched (4- and 5-positions) benzo(a)pyrene 4,5-oxide (BPO) by a purified glutathione transferase from little river skate (Raja erinacea) liver demonstrated that equivalent amounts of both the 4-glutathionyl (I) and 5-glutathionyl (II) BPO conjugates were formed. Separation of these conjugates by HPLC and subsequent ^{13}C NMR analysis revealed that the earlier eluting peak on the HPLC contained II and that the later eluting peak contained I. A significant feature is the production of I and II as single diastereoisomers, the implication being that each BPO enantiomer reacts with GSH enzymatically to produce only one of the two possible positional isomers. The chemically synthesized GSH conjugates of BPO consisted of all four stereoisomers. The additional diastereoisomers of I and II produced chemically emerge on the HPLC profile as a single peak, well resolved from the enzyme products. The reaction catalyzed by the little skate enzyme was further investigated using ^{3}H-BPO.

Similar experiments were conducted using purified rat liver glutathione transferases. With the rat enzymes isomer II again predominates over isomer I. However, the ratio of the isomers vary with the particular transferase. Incubations were conducted at various pH's. Although there was a slight change in the ratio of II:I, the differences were not considered significant.

The stereochemistry of the conjugation of glutathione with other arene oxides will be investigated. Studies using purified transferases from various sources similar to those described for benzo(a)pyrene 4,5-oxide will be conducted with phenanthrene 9,10-oxide, pyrene 4,5-oxide, benzanthracene 4,5-oxide and chrysene 4,5-oxide. Substrate concentrations, pH and ionic strength dependence of the conjugation will be investigated.

A parallel effort was initiated in order to develop procedures for the analysis, separation and purification of these thioether compounds. A separation technique, validated by the use of synthetic samples, would have immediate application in the analysis and identification of thioether metabolites from *in vitro* and *in vivo* experiments.

Separation of the thioether derivatives of styrene oxide by reverse phase-high pressure liquid chromatography (RP-HPLC) was optimal at acid pH, 3 or 4, or neutral. The separation was considerably faster at pH 7 albeit with some loss in resolution. An effect in the retention was observed resulting from the correlation between the distance of the charged groups and the non-polar groups within a molecule. A greater distance (glutathione) resulted in increased retention. In this manner the order of elution was, cysteine, cysteinylglycine, glutathione, and last eluting the N-acetylcysteine compounds. The separation of diastereomers, particularly the benzylic thioethers, was similarly explained on the basis of spatial interactions of charged and nonpolar groups and the relative hydrophobic surface available for binding to the RP-column as a result of these interactions. Both of these methods, synthetic and analytical, have already had a substantial impact on studies related to the metabolism of xenobiotics by the glutathione transferase enzyme system.

On the HPLC system, a correlation between the order of elution from RP-HPLC and the configuration at the site of attachment of sulfur will be sought by synthesizing representative glutathione conjugates from arene and alkene oxides. This type of stereochemical analysis has potential in determining stereochemical preferences in the formation of epoxides by the cytochrome P-450 system.

Presently, there is little or no information on this very important transformation. We have also explored the application of HPLC to the analysis and purification of peptides and proteins.

Glutathione S-transferase represents a family of enzymes involved in the detoxification of numerous xenobiotics by either catalyzing the conjugation of reduced glutathione with electrophiles or by functioning as the binding protein of bilirubin and carcinogens which do not serve as the substrate. Multiple forms of glutathione S-transferase from rat and human liver have been well characterized. These observations may indicate a species divergence of this class of enzymes as well as the species variations of its functional role as the detoxification enzymes. Therefore, independently we initiated detailed biochemical studies of glutathione S-transferases from mouse tissues in an attempt to resolve the following questions: 1) multiple forms of glutathione S-transferases and their distributions in mouse tissues; 2) substrate specificity and biochemical properties of the different forms of glutathione S-transferases as well as their functional implication; 3) immunological and structural relatedness of multiple forms of mouse glutathione S-transferases; 4) biochemical and immunological identity of microsomal glutathione S-transferases; and 5) elucidation of the molecular mechanism of action of glutathione S-transferases by NMR spectroscopy.

Three major forms of glutathione S-transferase (F1, F2 and F3) from mouse liver and one acidic form of testicular transferase (Ft) have been purified. Antisera were raised against each form of purified transferase. Immunologically, F1 and F2 transferase are virtually identical. The F3 transferase and F1 or F2 transferase are immunologically distinct. Ft transferase does not cross-react with F1 or F2 transferase, but partially cross-reacts with F3 transferase. F1 and F2 transferases have a homodimeric molecular weight of 44,000; whereas F3 and Ft transferases have homodimeric molecular weights of 50,000.

Substantial differences in structure and substrate specificity between mouse and rat or human transferases were noted. Immunologically and structurally, multiple forms of mouse liver transferase are mainly the product of two structural genes. This is in shart contrast to rat liver enzymes for which four immunological distinct forms of transferase were documented. In human liver, the five multiple forms of transferase are immunologically identical. This seems to suggest that genes controlling the expression of transferases are rapidly evolving. We have obtained about 100 mg each of the F2 and F3 transferases from mouse liver for NMR study. Preliminary results were obtained with respect to the binding of S(2,4-dinitrophenyl)-glutathione to mouse transferases. Currently we plan to use C^{13}-labeled reduced glutathione and substrates for more detailed investigation for the molecular mechanism of this important enzyme system for detoxification of numerous xenobiotics. Immunologically, we have demonstrated that mouse liver microsomal glutathione S-transferases have the same identity as the cytosolic enzyme. This suggests that microsomal transferases arise from the association between the microsomal membrane and the cytosolic transferases.

Two of the more important questions usually asked about the environmental impact of xenobiotics are what are the metabolites of a xenobiotic and how does it interact with the body. NMR spectroscopy is probably the singly most useful technique available to the chemist for determining the structure of organic compounds and for studying molecular interactions between a xenobiotic and biological materials. We have been using NMR methods to study problems of environmental significance. This has involved the application of existing NMR

techniques and evaluation of new techniques as they prove useful for solution of our particular problems.

NMR has been used extensively in our studies of the conjugation of glutathione with epoxides and the subsequent metabolism and excretion of these conjugates. ^{13}C NMR has been used to identify the glutathione conjugate isomers formed from styrene oxide, α-methylstyrene oxide, β-methylstyrene oxide, tetrahydronaphthylene 1,2-oxide and benzo(a)pyrene 4,5-oxide. Similar data have been obtained for phenanthrene 9,10-oxide and pyrene 4,5-oxide in anticipation of our future studies of the conjugation of these oxides. Data have also been obtained for the cysteinylglycine, cysteine and N-acetylcysteine (mercapturic acid) conjugates of styrene oxide. These data have permitted the development of rapid HPLC assays for these conjugates.

In anticipation of future studies of the metabolism of larger PAH's, the epoxide, cis- and trans-diols, quinone and mercapturic acid conjugates of acenaphthylene, phenanthrene, and pyrene have been examined by ^{13}C NMR spectroscopy as model compounds. The data obtained have been used to develop a set of substituent parameters for use in the identification of the oxidative metabolites of larger PAH's. By using these parameters, the chemical shifts of the metabolites of benzo(a)pyrene are predicted to a reasonable accuracy such that chemical shift assignments of these metabolites can be made. ^{13}C NMR chemical shift data have been obtained for the epoxides and diols of benzo(a)pyrene and for the isomeric hydroxybenzo(a)pyrenes.

^{1}H and ^{13}C NMR spectra of 1-, 4- and 9-azaphenanthrene, 4-azapyrene, 2-azafluoranthene and the benzocarbazoles have been obtained and chemical shifts and coupling constants extracted from the spectra. The data obtained for these compounds should prove useful in the identification of metabolites of nitrogen substituted PAHs.

There continues to be an interest at the Institute in polychlorinated aromatic hydrocarbons, particularly the chlorinated biphenyls and dioxins. One of the problems in working with these compounds is that they are usually present as mixtures of isomers and without authentic standards, the identification of the isomers present can prove difficult. We have looked at the effect of multiple chlorine substitution on NMR parameters to determine how useful additivity of chlorine substituent effects on NMR parameters will be in the identification of isomers. The ^{1}H and ^{13}C NMR spectra of all the chlorinated phenols have been obtained. Analysis of these spectra in terms of chemical shifts and coupling constants has allowed a set of additivity parameters for chlorine substitution on NMR parameters to be developed. It appears that additivity parameters accurately reproduce the proton-proton coupling constants obtained for ^{1}H NMR spectra and that this is a useful tool for determining the chlorine substitution pattern in polychlorinated aromatics of environmental interest. It is equally clear that additivity parameters are not useful for predicting the ^{1}H chemical shifts of polychlorinated aromatics. The additivity parameters for predicting ^{13}C chemical shifts appear to reproduce the chemical shifts with a reasonable degree of accuracy but it is doubtful if this alone would be enough to uniquely identify an unknown polychlorinated aromatic.

There is increasing evidence that certain metals produced as energy by-products may accumulate in body tissue and fluid and present serious health effects. Binding propensity of the heavy metals and toxicity and accumulation may be

correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them. Our studies have indicated that ^{207}Pb NMR studies would be useful as a sensitive probe into the binding of lead with biological materials and several of the factors influencing ^{207}Pb chemical shifts have been elucidated. Further studies have examined a number of organic ligand complexes of lead. In several cases, the exchange rate between "free and complexed" lead is in the intermediate range of rates such that the ^{207}Pb NMR signal is lost in the baseline, whereas in other cases, distinct resonances are observed. This loss of the resonance due to exchange rates will place a limitation on the usefulness of ^{207}Pb NMR for binding studies. As model systems, the binding of lead to crown ethers, thio- and azasubstituted crown ethers and amino acids has been examined.

The binding of copper and nickel to albumin has been investigated using ^{37}Cl NMR in collaboration with the Laboratory of Biophysics. There is a significant difference in the binding of these metals to human, bovine and dog serum albumin. In all cases, copper is more tightly bound than is nickel. Work is in progress to try to separate specific from non-specific binding.

OTHER ACTIVITIES

A substantial part of the overall efforts of these work groups involves collaborative/support activities. Collaborative projects can be initiated by individual scientists or may have Institute priority in terms of a "targeted research area" having immediate interest and seeking immediate data to bear on an imminent health problem. The routine support requests generally fall into one of two categories: short-term requests such as compound purity determination and organic synthesis by published procedures, and a variety of long-term requests which often require new and improved methodology development such as qualitative and quantitative analysis of complex mixtures and organic synthesis by unpublished procedures. In the latter case, it is difficult to determine the amount of time and effort which will be necessary to complete the task. A trial period may be necessary to demonstrate feasibility within the capabilities of the group. Therefore, for obvious reasons, these latter requests are carefully evaluated, and we frequently seek to do this work on a collaborative research support basis.

A GC/MS service is available to any member of the IRP staff who presents a reasonable request. This typically results in 2000-3000 data acquisitions per year with the majority being for the GC/MS analysis using electron impact ionization. Over the past two years we have upgraded the facility from low resolution GC/MS to high resolution GC/MS. Since bringing the exact mass measurement capability on stream we find approximately 60% of our service request now utilize this feature. We also maintain a group of mass spectrometrists eager to collaborate on interesting biomedical research problems.

Limited synthetic support is also available to IRP staff. Most of the effort in this area has been spent fullfilling requests from outside the Laboratory of Environmental Chemistry for the synthesis of high purity polychlorinated biphenyl isomers to support various testing studies. Other compounds prepared include mono- and di-demethyl-methoxychlor, a DDT derivative to support the radioimmunoassay program in the Laboratory of Environmental Chemistry, and several brominated naphthalenes and biphenyls.

Other work led to the synthesis and characterization of brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs. These include pentabromomethylnaphthalene, ^{14}C-labeled 1,2,4,6,7-pentabromonaphthalene and work toward modification of the 1,2,4,6,7-pentabromonaphthalene as a haptenic compound in radioimmunoassay work.

Requests for ^{1}H and ^{13}C NMR routine service for structure identification and confirmation have continued to increase during the past two years. This service will continue and should increase as more Institute investigators become aware of the potential of NMR. A Varian multi-nuclear FT-80 NMR spectrometer was installed in July, 1980 to provide additional NMR capabilities for routine service, collaborative support and research.

The exact molecular structures of some highly toxic polyhalogenated aromatic hydrocarbons have been obtained by X-ray crystallographic measurements or estimated based on postulated structures. The requirements of molecular symmetry and size as determined by the number, kind, and positions of halogens, planarity, interatomic distances and overall stereoelectronics suggest that a specific biological receptor may be involved which could account for the common toxic pattern. An underlying factor in the apparent symmetry requirement for biological activity and toxicity in these compounds appears to be net molecular polarizability. This property has been indirectly measured in a number of polychlorinated biphenyls and plans are underway to derive and calculate for a selected number of compounds their relevant electrostatic potential contour maps for comparison. The guinea pig will continue to be used as a screening animal model in providing direction for further biological and chemical study of related compounds. Additive or synergistic toxicity has also been demonstrated for these compounds. A molecular level binding event which correlates with in vivo potency is being sought.

A variety of contracts are also required to interact with the various research, collaborative/support components to maintain a viable program. Contractors are nearing completion of the analysis of large numbers of human milk, blood (serum) and placenta tissue samples for polychlorinated biphenyls, DDE and total organic chlorine content using newly developed and validated methods. Manuscripts are in preparation for the eventual publication of these methods. Other contract work aimed at providing rapid screening analysis of diverse sample matrices for aromatic hydrocarbon residues has afforded disappointing results and the work will be terminated. Contract work applying and developing procedures for the analytical determination of environmental chemicals by radioimmunoassay continues with renewed purpose and focus. A number of chlorinated dibenzo-p-dioxins are also being synthesized on contract as analytical standards or materials for toxicological testing purposes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10003-02 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Synthetic and Analytical Studies in Bioorganic Chemistry

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

PI:	O. Hernandez	Visiting Associate	LEC NIEHS
OTHER:	R.H. Cox	Research Chemist	LEC NIEHS
	K.K. Kohli	Visiting Fellow	LEC NIEHS
	A. Bhatia	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this project is to explore and develop synthetic and analytical methods for the study of biological processes. The nature of this methodology is determined by specific requirements from current projects within the Laboratory. In addition, expertise in bioorganic chemistry is reflected in consulting and collaborative activities with other research groups at the Institute.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: High pressure liquid chromatography (HPLC), carbon magnetic resonance, proton magnetic resonance, and the usual synthetic apparatus and equipment.

MAJOR FINDINGS AND PROPOSED COURSE: The HPLC method originally developed for the glutathione conjugates of styrene oxide was extended to include all the potential thioether metabolites of this model alkene oxide. Under the reversed-phase method used the order of elution of the conjugates was cysteine first, followed by cysteinylglycine, glutathione, and N-acetylcysteine compounds. A mechanistic model was proposed in order to explain the differences in chromatographic behavior of the various sulfur conjugates. In this model, the hydrophobic interactions between the reversed phase column and the sulfur conjugate(s) are modulated by the location of the charged (ionic) groups in the amino acid portion of the molecule. The distance and relative orientation of the charged species relative to the non-polar groups(s) may result in an increase or decrease of the hydrophobic surface available for binding. The HPLC experiments have stimulated further conformational studies on these compounds, as there seem to be intramolecular factors which prevail throughout a series of compounds, i.e. compounds having the same configuration elute in the same order.

Another aspect of HPLC as applied to biological problems was explored with the analysis of cytochrome P-450 samples. The mechanism of separation used was a combination of size exclusion and hydrophobic interactions. The cytochrome P-450 samples survived the HPLC conditions with recoveries in excess of 90%. Separation of the different forms of cytochrome P-450 was not possible under these conditions, however, we demonstrated that the level of induction by different inducers of cytochrome P-450 could be followed by monitoring absorbtion at 405 nm (heme chromophore) on the HPLC instrument.

Cytochrome P-420, the denatured form of cytochrome P-450, is easily separated and we obtained evidence for the presence of additional forms of P-420 and of the ease by which this protein forms high molecular aggregates. The HPLC conditions were successfully used for the removal of non-ionic detergents (Emulgen 911) from samples of cytochrome P-450.

The methods introduced for the protection of hydroxyl groups, as triphenylmethyl or silyl ethers, based on the use of 4-dimethylaminopyridine have become standard procedures in synthetic chemistry. The intermediate postulated in the triphenylmethylation reaction has been recently synthesized and characterized as 4-dimethylamino-N-triphenylmethylpyridinium chloride. This unusually stable N-triphenylmethyl pyridinium salt selectively derivatizes primary alcohols in the presence of secondary alcohols. In addition, it is possible to derivatize a nitrogen function in the presence of hydroxyl groups. This finding is of value for synthetic operations on polyfunctional molecules such as amino carbohydrate antibiotics, amino acids, and nucleosides. It also suggests ways of designing reagents for selective protein modification.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An active program in bioorganic chemistry contributes to maintain a high level of expertise in critically important areas such as biomechanisms, synthetic organic chemistry, and chromatographic techniques. The benefits of this approach are

reflected in consulting and collaborative activities with research groups throughout the Institute.

PUBLICATIONS

Hernandez, O., Yagen, B., Cox, R.H., Bend, J.R., and McKinney, J.D.: HPLC analysis of the isomeric thioether metabolites of styrene oxide. J. Liquid Chromatogr. (in press).

Hernandez, O., Chaudhary, S.K., Cox, R.H., and Porter, J.: Synthesis and characterization of 4-dimethylamino-N-triphenylmethyl pyridinium chloride: a postulated intermediate in the tritylation of alcohols. Tetrahedron Lett. 22, 1491-1494 (1981).

Kohli, K.K., Hernandez, O., and McKinney, J.D.: Fractionation by HPLC of microsomal cytochrome P-450 induced by hexachlorobiphenyl isomers. J. Liquid Chromatogr. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10004-02 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Nuclear Magnetic Resonance (NMR) Spectroscopy

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

PI:	R.H. Cox	Research Chemist	LEC NIEHS
OTHER:	O. Hernandez	Visiting Associate	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The silylation of functional groups plays an important role in making organic compounds more volatile for gas chromatography and mass spectroscopy and in protecting functional groups during further synthetic transformations. Using low temperature NMR, we have established that a complex is formed between a pyridine base and a silyl chloride. Equilibrium constants and thermodynamic parameters have been obtained for complex formation between various silyl chlorides and 4-substituted pyridines. This complex is probably the active silylating reagent and could explain why one functional group is selectively silylated in the presence of other similar functional groups.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{1}H and ^{13}C high-resolution nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Low temperature ^{1}H and ^{13}C NMR spectroscopy has been used to study the complex formation between pyridines and various silyl halides. Equilibrium constants and thermodynamic parameters have been obtained for complex formation between trimethylsilyl chloride, dimethyl-t-butylsilyl chloride, diphenyl-t-butylsilyl chloride and trimethylsilyl bromide and pyridine, 4-dimethylaminopyridine and 4-pyrrolidinopyridine. In no case was there evidence obtained for a complex between the silyl halides and pyridine. However, evidence for complex formation was obtained between the silyl halides and the 4-substituted pyridines. A steric effect was observed with the order of complex formation as follows: trimethylsilyl chloride>dimethyl-t-butylsilyl chloride>diphenyl-t-butylsilyl chloride. A solvent effect was also observed with complex formation greater in acetonitrile than in methylene chloride. The NMR evidence (^{1}H, ^{13}C and ^{29}Si) suggest that the complex is a N-silylpyridium halide salt and for the first time provides evidence for a complex as an intermediate in silylation reactions.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: NMR spectroscopy is probably the singly most useful technique available to the chemist for determining the structure of organic compounds and for studying molecular interactions. The data obtained during this reporting period should prove useful in our studies in the synthesis of metabolic intermediates of environmental significance.

PUBLICATIONS

Cox, R.H. and Sankar, S.: ^{1}H and ^{13}C NMR studies of 7-azaindole and related compounds. Org. Magn. Reson. 14, 150 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10005-02 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Pollutants on Immune Expulsion of Parasites

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

PI:	M.I. Luster	Research Microbiologist	LEC NIEHS
OTHER:	J.H. Dean	Research Microbiologist	EBB NIEHS
	G.A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The effects on immune elimination of helminthic parasites from mice exposed to various chemicals of environmental concern are being studied. Evaluation of adult worm longevity in exposed animals indicated that selected chemicals adversely affect the ability of treated hosts to respond normally to a helminthic infection. The T. spiralis model offers an extremely sensitive indicator for determining altered host susceptibility following chemical exposure and may play a role in increased human parasitic infection in persons exposed inadvertently to selected chemicals.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: In addition to the T. spiralis model now completed, lethal and non-lethal strains of Plasmodium (berghi and yoelii) are being evaluated for potential implementation into a more extensive host-resistance panel. Parameters being examined include time to death following infection, progression of parasitemia and anemia.

MAJOR FINDINGS AND PROPOSED COURSE: These studies clearly demonstrate that expulsion of T. spiralis adults from the gut offers an extremely sensitive endpoint to assess host resistance following chemical exposure in mice. We were also able to demonstrate that this model is dependent upon cell mediated T-cell immunity with a minor but significant contribution by B-lymphocytes and macrophages. Enumeration of muscle larvae does not offer a sensitive endpoint. Studies are now in progress to examine Plasmodium as a sensitive and simple host resistance model. Preliminary data has suggested that following the progression of parasitemia offers a sensitive endpoint.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the still relatively high incidence and prevalence of parasitic disease in certain areas, evaluation of the effects of environmental contaminants on the course and severity of parasitic infection is relevant on a regional and global scale. An evaluation is being made of the above mentioned assays to compare their sensitivity with assays of host potential as useful tests of host resistance.

PUBLICATIONS

Dean, J.H., Luster, M.I., Boorman, G.A., Padarathsingh, M.L. and Leubke, R.W.: Host resistance models as an endpoint for assessing immune alterations following chemical exposure. In Dean, J.H. and Padarathsingh, M.L. (Ed.): The Biological Relevance of Immunosuppression. Van Norstrand Reinhold, New York, 1981.

Luebke, R.W., Luster, M.I. and Dean, J.H.: Effects of subchronic adult exposure to diethylstilbestrol on the immune response of mice to Trichinella spiralis infection. Infect. Immun. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10006-02 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Diethylstilbestrol on the Immune System

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

PI:	M.I. Luster	Research Microbiologist	LEC NIEHS
OTHER:	G.A. Boorman	Research Pathologist	EBB NIEHS
	J.H. Dean	Research Microbiologist	EBB NIEHS
	M. Deiter	Chemical Manager	NTP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The DES induced immunosuppression has been described in the previous annual report. We are presently examining the mechanisms of DES-induced immunosuppression at the cellular and molecular levels by examining suppressor substance produced by macrophages of DES treated animals and various changes in macrophage enzymes and alterations in metabolic pathways.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mechanisms are being examined by a variety of in vitro assays including lymphoproliferative responses and macrophage cytostasis. In addition, a variety of nucleoside enzymes, glycloysis, etc. are being evaluated in vitro by spectrophotometric and fluorometric techniques.

MAJOR FINDINGS AND PROPOSED COURSE: These studies indicated that pharmacologically relevant doses of DES (used therapeutically in humans) can suppress specific immunity while augmenting macrophage activity. This immune suppression can result from suppressor factors produced by activated macrophages and is analagous to many immunosuppressed cancer patients. Like cancer patients, this suppressor substance can be inhibited by indomethacin and is produced by macrophages. Thus, it is likely to be prostaglandin in nature. Enzyme studies have shown that while most enzyme activity is increased that several enzymes are markedly depressed suggesting that these are involved in bacterial killing.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: DES treatment in humans and laboratory animals has been associated with tumor development. Recently, immunosuppression has been reported to occur in humans treated with DES for prostatic cancer. We are attempting to determine the relationship of DES associated cancers and immunosuppression.

PUBLICATIONS

Boorman, G.A., Luster, M.I., Dean, J.H. and Wilson, R.E.: The effect of adult exposure to diethylstilbestrol in the mouse on macrophage functions and numbers. J. Reticuloendothel. Soc. 28: 547, 1980.

Luster, M.I., Boorman, G.A., Dean, J.H., Luebke, R.W. and Lawson, L.D.: The effect of adult exposure to diethylstilbestrol in the mouse: Alterations in immunological functions. J. Reticuloendothel. Soc. 28: 561-570, 1980.

Dean, J.H., Luster, M.I., Boorman, G.A., Luebke, R.E. and Lauer, L.D.: The effect of adult exposure to diethylstilbestrol in the mouse: Alterations in tumor susceptibility and host resistance parameters. J. Reticuloendothel. Soc. 28: 571-583, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10007-01 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
High Pressure Liquid Chromatography/Mass Spectrometry

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

PI:	C.E. Parker	Research Chemist	LEC NIEHS
OTHER:	R. Hall	Engineer	RSB NIEHS
	J.R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)
Research Services Branch

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A positive/negative chemical ionization mass spectrometer has been coupled to a liquid chromatograph via a HPLC/MS variable split-type interface. The system is being further refined and tested for its use in analysis of thermally labile compounds.

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 accommodate a Hewlett-Packard HPLC/MS variable split-type interface.

MAJOR FINDINGS AND PROPOSED COURSE: The system is now working in both positive and negative ion chemical ionization modes. Work is continuing to determine the effect of source geometry, temperature, pressure and LC solvent systems on sensitivity and specificity. Work is also proceeding on a similar interface for LC/MS/MS.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10008-01 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemical Analysis of Bovine Tissues for Pentachlorophenol and Related Compounds

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

PI:	C.E. Parker	Research Chemist	LEC NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	H.B. Matthews	Research Chemist	LP NIEHS
	J.R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

Environmental Biology Branch
Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project was part of a cattle feeding study to distinguish between the toxicity of pentachlorophenol and the toxicity of its impurities. Qualitative and quantitative determinations of the chlorinated dibenzo-p-dioxin content of selected tissues and body fluids were performed.

PROJECT DESCRIPTION

METHODS EMPLOYED: The toxicopathological effects of technical (t) and analytical (a) grade pentachlorophenol (PCP) were compared in female yearling Holstein cattle. Four groups of three calves each were exposed for 160 days to aPCP, or a mixture thereof in the feed. A fifth group of three animals served as unexposed controls. All treated cattle received the same amount of PCP; 20 mg/kg/day for 42 days followed by 15 mg/kg/day for the remainder of the study. The four doses fed were 100% aPCP, 10% tPCP + 90% aPCP, 35% tPCP + 65% aPCP, and 100% tPCP. At the end of the study, samples of liver and adipose tissue were analyzed at NIEHS for chlorinated dibenzo-p-dioxin and furan content by GC/MS, and blood was assayed for PCP and hexachlorobenzene (HCB) by ECGC (under contract).

MAJOR FINDINGS AND PROPOSED COURSE: Analyses indicated increases in the dioxin and furan concentrations of liver and adipose tissue which were correlated with the concentration of tPCP in the diet. The liver contained markedly higher dioxin levels than the adipose tissue. Blood HCB levels also increased as the dose of tPCP increased, while blood PCP levels were lowest in the cows fed the highest tPCP dose. This project has been completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project was designed to determine whether exposure of cattle to technical pentachlorophenol (from treated wood, for example) might be a mechanism by which dioxins and furans could enter the human food chain.

PUBLICATIONS

Parker, C.E., Jones, W.A., Matthews, H.B., McConnell, E.E. and Hass, J.R.: The chronic toxicity of technical and analytical pentachlorophenol in cattle. II. Chemical analyses of tissues. Toxicol. Appl. Pharmacol. 55, 359-369 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10009-01 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mouse Glutathione S-Transferases

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

PI:	C.Y. Lee	Senior Staff Fellow	LEC NIEHS
OTHER:	R.H. Cox	Research Chemist	LEC NIEHS
	J.D. McKinney	Supervisory Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Four different forms of mouse glutathione S-transferases were purified. F1, F2 and F3 transferases were purified from mouse liver by ammonium sulfate fractionations, DEAE-cellulose, CM-cellulose and hydroxyapatite chromatography. F transferase from mouse testis was partially purified from mouse testis by ammonium sulfate fractionations DEAE cellulose and hydroxyapatite chromatography and isoelectric focusing. Antisera were raised in rabbits against each form of transferase. The following biochemical parameters were determined for each mouse transferase: (a) native and subunit molecular weight; (b) Km for 1-chloro-2,4-dinitrobenzene and GSH; (c) isoelectric point; (d) Ki for S-(2,4-dinitrophenyl)glutathione; (e) immunological cross reactivity among different forms of transferase; (f) thermal stability and pH-dependent activity; (g) amino acid composition and peptide mapping for liver F1, F2 and F3 transferases.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Conventional procedures were employed for enzyme purification. Steady-state kinetics was studied spectrophotometrically. A standard procedure for immunization was used for raising antisera in rabbits against each form of glutathione S-transferase.

MAJOR FINDINGS AND PROPOSED COURSE: Mouse liver F1 and F2 transferase were shown to be homodimers with a native molecular weight of 44,000, whereas liver F3 and testis Ft transferase were homodimers with a native molecular weight of 50,000. F1 transferase is immunologically identical to F2 transferase but is distinct from F3 or Ft transferase. Antiserum to F3 transferase partially cross-reacts with Ft transferase, but no cross-reactivity was observed between the antiserum to Ft transferase and F3 transferase. The isoelectric points were determined to be 6.5 $\pm$ 0.5, 8.2, 8.8 and 5.0, respectively for F1, F2, F3 and Ft transferase. Different forms of mouse transferase exhibit quite similar biochemical properties. Compared to glutathione S-transferases from rat liver, large species variations of this enzyme system were observed, not only in terms of their substrate specificity, but also the presence of different multiple forms.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Glutathione S-transferase is known to be an important enzyme system for the detoxification of numerous xenobiotics. Large species variations of this enzyme system (substrate specificity and multiple forms) may become an important concern in choosing a proper animal species for evaluations of biochemical transformation and toxicity of xenobiotics. Our study on mouse glutathione S-transferases indicated a large species diversity for this enzyme system.

PUBLICATIONS

Lee, C.-Y., Johnson, L., Cox, R.H., McKinney, J.D. and Lee, S.-M.: Mouse liver glutathione S-transferases. J. Biol. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 10010-01 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Carcinogens on the Immune System

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

PI:	M.I. Luster	Research Microbiologist	LEC NIEHS
OTHER:	J.H. Dean	Research Microbiologist	EBB NIEHS
	G.A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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 (200 words or less - underline keywords)

The relationship of various carcinogens and structurally similar non-carcinogens are being examined in relationship to immune alteration. We have examined benzo(a)pyrene and benzo(e)pyrene as well and methyl and ethyl carbamate pairs. These studies have shown that B-cell but not T-cell function are impaired by the carcinogens but not the non-carcinogens. Urethane (ethyl carbamate) appears to have a preferential affect on the bone marrow. Myelotoxicity can be demonstrated far below levels which induce general toxicity or immunotoxicity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Immune functions and host resistance was examined using a large battery of assays. These include assays for lymphocyte, bone marrow and macrophage functions, pathotoxicology and resistance to tumor cell challenge and infectious agents.

MAJOR FINDINGS AND PROPOSED COURSE: These studies indicate that non-toxic dosages of environmental carcinogens but not their noncarcinogenic congeners have a detrimental effect on B-cell immunity and bone marrow function but not host resistance (T-cell type) or cell mediated immunity. It appears unlikely that immunosuppression induced by these carcinogens is responsible for their increased carcinogenicity. Various studies are going to be conducted with a more potent carcinogen (DMBA) as well as tumor promotors (phorbol esters) in order to expand on these initial studies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many polycyclic hydrocarbons that are considered environmental pollutants are toxic and more importantly carcinogenic, being primarily involved in tumor initiation. Since loss of immunocompetence in humans and laboratory animals can be related, in many cases, to increased cancer risk, it is important to establish the nature and mode of action, if any, these chemicals exert on the immune system. Once this has been established in laboratory animals, it will facilitate human risk assessment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30003-09 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Analytical Methodology

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

PI:	P.W. Albro	Research Chemist	LEC NIEHS
OTHER:	J.R. Hass	Research Chemist	LEC NIEHS
	K. Chae	Chemist	LEC NIEHS
	Y. Tondeur	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This general project area has as its objective, to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Recent work has emphasized development and validation of methods for (a) quantitative and reproducible extraction and clean-up of halogenated aromatic compounds from soil and adipose tissue for subsequent GC-MS or immunoassay, and (b) fortifying samples under conditions yielding equilibration of "spike" with endogenous compound. In addition, applications of capillary column gas chromatography and two-dimensional gas chromatography are under active investigation.

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: Methods have been developed for the analysis of extremely complex mixtures of halogenated aromatics (such as transformer fluids), and for the complete analytical separation of all PCB isomers. Studies on the cleanup recoveries of dioxins at the picogram level have led to much better understanding of the recovery variabilities in the literature, but as yet no solutions to the problems. Since much of the effort in this project area is in response to expressed needs of the Institute, future efforts can not be predicted in detail.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Laboratory of Chemistry as well as be of utility for other studies within the Institute.

PUBLICATIONS

Albro, P.W., and Parker, C.E.: General approach to the fractionation and class determination of complex mixtures of halogenated aromatic compounds. J. Chromatogr. 197: 155, 1980.

Parker, C.E., and Albro, P.W.: Quantitation of halogenated aromatic compounds. Annual meeting of the American Society for Mass Spectrometry, 1981.

Albro, P.W.: Validation of extraction and cleanup procedures for environmental analysis. in Environmental Health Chemistry: Chemistry of Environmental Agents as Potential Human Hazards. J.D. McKinney (ed.), Ann Arbor Science Publ. Co., Ann Arbor, MI, 1981, pp. 163-175.

Albro, P.W., Corbett, J.T., and Schroeder, J.L.: Quantitative characterization of polychlorinated biphenyl (PCB) mixtures by gas chromatography using capillary columns: Aroclors® 1248, 1254 and 1260. J. Chromatogr. 205: 103 (1981).

Tondeur, Y., Hass, J.R., Albro, P.W. and Chae, K.: Quantitative HRGC-HRMS: Study on the use of a non-isotopically labeled internal standard in the analysis of 2,3,7,8-TCDD in environmental samples. Annual meeting of the American Society for Mass Spectrometry, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30015-07 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Chemical Ionization Mass Spectrometry

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

PI: J. R. Hass — Research Chemist — LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)

M.M. Bursey, Department of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The negative chemical ionization (NCI) mass spectra of selected S-triazine herbicides were studied. All compounds exhibited intense $(M-H)^-$ and fragmentation reactions characteristic of structural features. The NCI mass spectrometer was modified for combined high pressure liquid chromatography/mass spectrometry as discussed in report Z01 ES 10007-01 LEC.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromatography/mass spectrometry techniques, negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: MENI spectra showed the following for compounds containing only Cl (I-V,VII,IX): 1) Cl ions (intensity >40%). 2) $(M-H^{\cdot})^{-}$ (intensity >60% except VII (36%) and IX (10%)). 3) Loss of mass 36 and 37 ($M-H^{\cdot}-Cl^{\cdot}$ and $M-2H^{\cdot}-Cl$) except IX (no mass loss of 36). 4) Loss of $Cl^{\cdot}$ (II-V&VII). 5) Loss of alkyl group, only, not observed (intensity 40%). 6) Loss of $H^{\cdot}+Cl^{\cdot}$ followed by loss of an alkyl group (except VII); this loss forms: a) II-V&IX was the base peak. b) R=isopropyl loss preferred 5 to 1 over the cyclopropyl loss (III). c) R=isopropyl preferred 4 to 1 over ethyl (V). 7) M/Z=66.0088, C_2N_3, present (except VII). MENI spectra for compounds containing Cl and CN (VI-VII) showed similar characteristics to the above results as well as the following: a) An $M^{-\cdot}$ observed $[(M-H^{\cdot})^{-}>M^{-\cdot}]$. b) No loss of $Cl^{\cdot}$. c) Loss of alkyl group observed (VI, 7%; VII, 32%). d) M/z=66 observed in VIII (4.1%) but not in VI. 7) Formation of $(M+Cl)^{-}$.

MIKES analysis showed no unimolecular decompositions of the $(M-H^{\cdot})^{-}$ for the four compounds studied (I-IV). Negative/negative CID/MIKES for the $(M-H^{\cdot})^{-}$ indicate the following: 1) Prominent losses involving $H^{\cdot}$ and $2H^{\cdot}$ (with the exception of II). 2) Loss of the alkyl group (III, loss of isopropyl preferred over cyclopropyl). 3) In all cases, one observed loss of $^{\cdot}CH_3$ and/or $^{\cdot}CH_3 + xH^{\cdot}$ (x=1-3). 4) In I, II, and IV, one observes loss of $Cl^{\cdot}$ or $2H^{\cdot} + Cl^{\cdot}$.

Predioxins were found to give negative ion reactions similar to those previously observed for dioxins.

This project will be extended by the study of CI spectra of other environmental hazards.

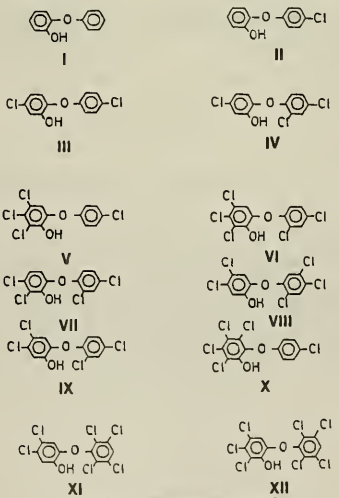

FIGURE 1. Compounds studied.

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 will lead to better methods of structural identification of organic contaminants 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

Busch, K.L., Norstrom, A., Nilsson, C.A., Bursey, M.M. and Hass, J.R.: Negative ion mass spectra of some polychlorinated 2-phenoxyphenols. Environ. Health Persp. 36, 1125-1134 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30018-08 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Ester Hydrolases at the Environmental Interface

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

PI: P. W. Albro Research Chemist LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to evaluate the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. These enzymes are also used as models in studies of protein-ligand interactions. Through studies of the kinetic isotope effects in D_2O we have been able to distinguish between general base catalysis and nucleophilic enhancement as the mechanism of the nonspecific lipase from rat pancreas.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic rubrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbamates.

MAJOR FINDINGS AND PROPOSED COURSE: Kinetic studies in D_2O indicate that the mechanism for catalysis by pancreatic non-specific lipase is general base. Inhibition/activation of this enzyme by salts is complex, non-linear, and difficult to distinguish from effects of salts on micellarization of substrate. The enzyme is a useful laboratory reagent, permitting the hydrolysis to completion of esters of primary or secondary alcohols, or phenols, at neutral pH. No specific future studies are planned at this time.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never previously been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30020-10 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Transport and Metabolism of Phthalate Esters

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

PI:	P. W. Albro	Research Chemist	LEC NIEHS
OTHER:	J. R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)
C. C. Peck, M.D., LTC, MC, USUHS

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SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Chemical dynamic studies have detected two thresholds in the intestinal absorption and transport of DEHP in rats. Above a single oral dose of 200 mg/Kg body weight, intact DEHP passes through unabsorbed. Above 470 mg/Kg, unhydrolyzed DEHP is absorbed and reaches the liver. The alcohol moiety of DEHP can covalently bind to DNA. Rats do not metabolize DEHP in a manner comparable to man; rats are not considered an acceptable animal model for possible toxic effects of DEHP in humans.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques, HPLC.

MAJOR FINDINGS AND PROPOSED COURSE: Di-(2-ethylhexyl)phthalate, a reported hepatocarcinogen in rats and mice, is metabolized differently in rats and primates. Rats produce highly oxidized, free metabolites while primates produce glucuronide esters of less-oxidized metabolites. In rats, the ethylhexyl moiety or part thereof can covalently bind to DNA, although no such binding occurs when free 2-ethylhexanol is given. Oral administration of DEHP to rats does not result in exposure of the liver to intact diester until the oral dose exceeds 470 mg/Kg body weight. Future studies will include further delineation of the requirements for covalent binding to DNA, and identification of the metabolites in additional species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PUBLICATIONS

Harvan, D.J., Hass, J.R., Albro, P.W., and Friesen, M.D.: Mass spectrometry of di-(2-ethylhexyl)phthalate metabolites. Biomed. Mass Spec. 7: 242, 1980.

Albro, P.W., Hass, J.R., Peck, C.C., Odom, D.G., Corbett, J.R., Bailey, F.J., Blatt, H.E., and Barrett, B.B.: Identification of the metabolites of di-(2-ethylhexyl)phthalate in urine from the African Green Monkey. Drug Metab. Disp. (in press).

Albro, P.W., Corbett, J.T., Schroeder, J., Jordan, S., and Matthews, H.B.: The fate of di-(2-ethylhexyl)phthalate in rats. The Toxicologist 1: 55, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30034-06 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemistry of Aromatic Compounds and Their Environmental Transformation Products

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

PI:	L. A. Levy	Research Chemist	LEC NIEHS
OTHER:	S. Kumar	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

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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 (200 words or less - underline keywords)

The development of rational synthetic routes to polynuclear aromatic hydrocarbons and their metabolites have been investigated. Models appropriate to the study of the chemical and physical properties of these classes of compounds as potential persistent environmental agents have been prepared.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, nuclear magnetic resonance spectroscopy, other spectroscopic methods (IR, UV), chromatography (column, glc, hplc).

MAJOR FINDINGS AND PROPOSED COURSE: The new methodology developed for the synthesis of benzanthracenes has been broadened and extended to prepare more complex examples of polynuclear aromatic hydrocarbons. These have included chrysenes and chrysene derivatives, dibenzanthracenes, cyclopentobenzanthracenes and hetero substituted benzanthracene derivatives. The activity of some of these new compounds have been determined and are being correlated with their structural parameters.

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. The activity of new synthetic PAH will be evaluated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polynuclear aromatic hydrocarbons are recognized to be of major environmental importance due to their widespread ecological distribution and, in some cases, their concentration in the food chain. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit critical biological and toxicological studies. Synthetic availability of appropriate model compounds allows further examination of the mechanisms of their biological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30041-06 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Hydrophobic Haptens

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

PI:	P. W. Albro	Research Chemist	LEC NIEHS
OTHER:	M. I. Luster	Research Microbiologist	LEC NIEHS
	K. Chae	Chemist	LEC NIEHS
	J. D. McKinney	Supervisory Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, CMB

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Radioimmunoassays for chlorinated dibenzo-p-dioxins, -biphenyls, and -dibenzofurans have been developed, as has a radioimmunoassay for mono-2-ethylhexyl phthalate in plasma. Development of a radioimmunoassay for 2,4,5-trichlorophenoxyacetic acid has encountered unexpected difficulties. A contract effort for validation and refinement of these procedures has been initiated. The reliability (95% confidence limits) of the dioxin assay has been evaluated for human adipose tissue.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques: condensation reactions, amination, reduction, mixed ester formation, active ester formation, convergent synthesis, spectroscopic and chromatographic characterization. Conjugation techniques: diazo coupling, mixed anhydride acylation, active ester acylation. Characterization of conjugates by UV spectroscopy, gel filtration, amino acid analysis, NMR spectrometry, and chemical assay of functional groups. Antibody production methods: standard procedures, with adjuvant, in rabbits. Antibody assay methods: double immunodiffusion, fluorescent antigen, radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: Although several previous attempts to develop RIAs for 2-ring halogenated aromatic compounds have been successful, we have been unable to generate antibodies in rabbits that can recognize 2,4,5-trichlorophenoxyacetic acid or its derivatives. Extreme animal variability in antibody specificity to dioxins seems to be genetically determined. The dioxin RIA give 95% detectability (freedom from false negatives) down to 100 pg of 2,3,7,8-TCDD in human fat. Future studies will involve the development of RIAs for additional compounds as well as evaluation of non-analytical applications of these antibodies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Luster, M.I., Albro, P.W., and McKinney, J.D.: Analysis of TCDD in human tissues by RIA. The Toxicologist 1: 240, 1981.

McKinney, J.D., Albro, P.W., Luster, M.I., Corbett, B.J., Schroeder, J.D., and Lawson, L.D.: Development and reliability of a radioimmunoassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Anal. Chem. Workshop, Rome, Italy, October, 1980.

Luster, M.I., Albro, P.W., Chae, K., Chaudhary, S.K., and McKinney, J.D.: Development of radioimmunoassays for chlorinated aromatic hydrocarbons. in The Chemistry of Environmental Agents as Potential Human Hazards, J.D. McKinney (ed.), Ann Arbor Science Publ., Inc., Ann Arbor, MI (1980) pp. 279-297.

Luster, M.I., Albro, P.W., Chae, K., Lawson, L.D., Corbett, J.T., and McKinney, J.D.: Radioimmunoassay for quantitation of 2,3,7,8-tetrachlorodibenzofuran. Anal. Chem. 52: 1497, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30050-05 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemical and Enzymatic Conjugation of Glutathione with Arene Oxides and Epoxides

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

PI:	O. Hernandez	Visiting Scientist	LEC NIEHS
OTHER:	R.H. Cox	Research Chemist	LEC NIEHS
	J. Bend	Research Pharmacologist	LP NIEHS
	A. Bhatia	Visiting Fellow	LEC NIEHS

COOPERATING UNITS (if any)

Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. N01-CP-33387. Supplemented by NIEHS)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.9	1.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The chemical conjugation of specific benzo[a]pyrene oxides and other epoxides to glutathione, enzymatically and non-enzymatically, is being investigated using nuclear magnetic resonance (NMR) spectroscopy, chemical synthesis, and high pressure liquid chromatography (HPLC). The regiospecificity and stereospecificity of the conjugation reaction is being determined. A HPLC assay has been developed for the glutathione conjugates of benzo[a]pyrene 4,5-oxide and the conjugation with various purified glutathione S-transferases examined for stereochemical detail. The stereochemistry of the isomers of the glutathione and mercapturic acid conjugates of styrene oxide has been established.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy; high pressure liquid chromatography (HPLC); organic syntheses, in vitro biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: The initial studies of the conjugation reaction of glutathione with (+)-benzo[a]pyrene 4,5-oxide have provided the basis for a new approach to biomechanisms involving the glutathione transferases. This approach is based on the stereochemical analysis of the conjugate products. The stereochemical profiles generated by the enzyme mediated reactions are markedly different from those found in the chemical reaction. Thus, a qualitative and quantitative distinction is easily established. Further work with purified glutathione transferases and (+)-benzo[a]pyrene 4,5-oxide has offered the following observations: (1) the ability of a glutathione transferase to catalyze the reaction of glutathione with (+)-benzo[a]pyrene 4,5-oxide would appear to be related to its pI, those proteins with low pI are more efficient, (2) a common feature of the glutathione transferases examined up to this point is the initial preferential formation of only one stereoisomer of the conjugate product. The implication being that one enantiomer of (+)-benzo[a]pyrene 4,5-oxide is preferentially metabolized by the enzymes. The enantioselectivity of this reaction is presently being pursued by the use of optically pure benzo[a]pyrene 4,5-oxide.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanistic aspects of the GSH transferase reaction underscore the relevance of stereochemical factors in influencing the rate of elimination of enantiomeric oxides via the glutathione pathway.

PUBLICATIONS

Yagen, B., Hernandez, O., Bend, J.R. and Cox, R.H.: Synthesis and relative stereochemistry of the four mercapturic acids derived from styrene oxide and N-acetylcysteine. Chem.-Biol. Interact. 34, 57-67 (1981).

Hernandez, O., Walker, M., Cox, R.H., Foureman, G.L., Smith, B.R. and Bend, J.R.: Regiospecificity and stereospecificity in the enzymatic conjugation of glutathione with (+)-benzo(a)pyrene 4,5-oxide. Biochem. Biophys. Res. Commun. 96, 1494-1502 (1980).

Cox, R.H., Hernandez, O., Yagen, B., Smith, B., Bend, J.R. and McKinney, J.D.: ^{13}C NMR studies of the structure and stereochemistry of products derived from glutathione transferase reactions with epoxides. In McKinney, J.D. (Ed.): The Chemistry of Environmental Agents as Potential Human Hazards. Ann Arbor Science, Ann Arbor, Michigan, 1980, pp. 403-423.

Hernandez, O., Yagen, B., Cox, R.H., Smith, B.R., Foureman, G., Bend, J.R. and McKinney, J.D.: Stereospecificity and regioselectivity in the reaction of epoxides with glutathione. In McKinney, J.D. (Ed.): The Chemistry of Environmental Agents as Potential Human Hazards. Ann Arbor Science, Ann Arbor, Michigan, 1980, pp. 425-444.

Hernandez, O., Foureman, G.L., Cox, R.H., Bend, J.R., Walker, M., and Smith, B.R.: Stereo- and regioselectivity in the enzymatic conjugation of glutathione with (±)-benzo[a]pyrene 4,5-oxide. International Symposium on Polynuclear Aromatic Hydrocarbons, Battelle Laboratories, Columbus, Ohio (in press).

Yagen, B., Hernandez, O., Bend, J.R., and Cox, R.H.: Synthesis and relative stereochemistry of the benzylic thioether diastereomers formed from glutathione and styrene oxides. Bioorg. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30051-05 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of Specific Binding Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons

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

PI:	J.D. McKinney	Supervisory Research Chemist	LEC NIEHS
OTHER:	P.W. Albro	Research Chemist	LEC NIEHS
	K. Chae	Research Chemist	LEC NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The exact molecular structures of some highly toxic polyhalogenated aromatic hydrocarbons have been obtained by X-ray crystallographic measurements or estimated based on postulated structures. The requirements of molecular symmetry and size as determined by the number, kind, and positions of halogens, planarity, interatomic distances and overall stereoelectronics suggest that a specific biological receptor may be involved which could account for the common toxic pattern. An underlying factor in the apparent symmetry requirement for biological activity and toxicity in these compounds appears to be net molecular polarizability. The guinea pig will continue to be used as a screening animal model in providing direction for further biological study of related compounds. A molecular level binding event which correlates with in vivo potency is being sought.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic methods along with X-ray crystallography and other methods for measuring physical/chemical properties with associated equipment and techniques was used primarily in this phase of the work. Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy using specifically 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.

MAJOR FINDINGS AND PROPOSED COURSE: The guinea pig was used as an extremely sensitive animal model to investigate toxic effects of certain halogenated aromatic hydrocarbons. These compounds include members of the biphenyl, naphthalene, dibenzofuran and dibenzo-p-dioxin classes and other related compounds. Highly toxic members of each of these classes were found with bromine substitution required on the naphthalene nucleus.

Work is continuing to examine the significance of the brominated naphthalenes in the toxicity of polybrominated biphenyl (PBB) mixtures. Representative brominated naphthalenes in PBBs were found to be highly toxic in guinea pigs in contrast to pure brominated biphenyls. One of the major pentabrominated naphthalenes found in PBBs was significantly more toxic to the guinea pig in the presence than in the absence of PBBs suggesting an additive or synergistic effect. The brominated methylnaphthalenes are being studied as possible additive agents in PBBs.

Presently, used assays for the "cytoplasmic receptor" that binds TCDD are either extremely inaccurate (requiring correction for 98 + % nonspecific binding) or extremely tedious (requiring sucrose gradient centrifugation or isoelectric focusing). Making use of the principles developed for radioimmunoassay of TCDD, we have devised a binding assay for the receptor that has relatively little nonspecific binding and therefore requires no extremely elaborate correction procedures. We have begun to use this assay in the purification of the receptor.

Future work will study the ability of TCDD antibodies and other selected proteins to compete with [cytoplasmic receptor for P-448 induction] for binding these inducers. We also hope to compare the antibody site to the receptor binding site. Affinity chromatography using receptor-binding analogs of TCDD coupled to a solid support will be investigated as a means of isolating the receptor. Other binding proteins will be examined.

Metabolic events caused by TCDD have been detected within hours after dosing in exploratory studies. In general, these events represent the initiation of possible self-perpetuating reactions, possibly involving free radicals. Qualitative differences in the early response of rats to sublethal and lethal doses have been seen in various aspects of lipid metabolism.

The qualitative ability of various PCB isomers to serve as inducers of cytochrome P-450-dependent, cytochrome P-448-dependent, both types or neither type of mixed function oxidase activity has been correlated with the molar polarizability of the PCB isomers in a manner permitting the prediction of activity of untested

isomers. The freedom from a requirement for metabolism has been shown. Since PCBs may be the simplest molecular structures showing these few types of activity, this approach provides the potential of visualizing the initial step in the induction process. Molecular polarization studies will be applied to carcinogenic and noncarcinogenic polynuclear aromatic hydrocarbons to assess the generality of the predictive approach.

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. and Singh, P.: Structure activity relationships in halogenated biphenyls: Unifying hypothesis for structural specificity. Chem.-Biol. Interact. 33: 271, 1980.

Albro, P.W. and McKinney, J.D.: The relationship between polarizability of PCBs and their induction of mixed function oxidase activity: Chem.-Biol. Interact. 34: 373, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30060-04 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Structure of Fungal Metabolites

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

PI: R.H. Cox Research Chemist LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)
National Peanut Laboratory, Dawson, Georgia
U.S.D.A., Georgia-South Carolina Area, Tifton, Georgia

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

^{1}H and ^{13}C nuclear magnetic resonance (NMR) is being used to characterize toxic fungal metabolites. New compounds whose structures were determined include three new cytocholasins related to cytochalasin H from Phomopis sp., dihydropergillin from A. ustus several new trichothecenes related to T-2 toxin.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Most of the compounds investigated in this project were either toxic and/or exhibited significant plant growth regulating properties. This project is being terminated due to the resignation of the principal investigator.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Molds found growing on cereal crops in many instances produce toxic fungal metabolites that may possibly ultimately end up in the human food chain (i.e. the aflatoxins). Therefore, identification of these metabolites and screening for biological activity are needed. Furthermore, it is likely that some of the metabolites may possess biological activity beneficial to man.

PUBLICATIONS

Dorner, J.W., Cole, R.J., Springer, J.P., Cox, R.H., Cutler, H., and Wicklow, D.: Isolation and identification of two new biologically active norditerpene dilactones from Aspergillus wentii. Phytochem. 19, 1157 (1980).

Springer, J.P., Cox, R.H., Cutler, H.G. and Crumley, F.G.: The structure of chaetoglobosin K. Tetrahedron Lett. 21, 1905 (1980).

Cole, R.J., Stuart, B.P., Lansden, J.A. and Cox, R.H.: Isolation and redefinition of the toxic agent from cocklebur (Xanthium strumarium). J. Agric. Food Chem. 28, 1330 (1980).

Cole, R.J., Dorner, J.W., Springer, J.P. and Cox, R.H.: Indole metabolites from a species of Aspergillus Flavus. J. Agric. Food Chem. (in press).

Cutler, H.G., Crumley, F.G., Springer, J.P., Cox, R.H., Cole, R.J., Dorner, J.W. and Thean, J.E.: Pergillin: A nontoxic fungal metabolite with moderate plant growth inhibiting properties from Aspergillus ustus. J. Agric. Food Chem. 28, 989 (1980).

Yagen, B., Horn, P., Joffe, A.Z., and Cox, R.H.: Isolation and structure elucidation of a novel sterol metabolite of Fusarium sporotrichioides 921. J. Chem. Soc., Perkin Trans. I. 2914 (1980).

Dorner, J.W., Cole, R.J., Hill, R., Wicklow, D. and Cox, R.H.: Penicillium rubrum and P. biforme, new sources of rugulovasines A and B. Appl. Environ. Micro. 40, 685 (1980).

Cole, R.J. and Cox, R.H.: Handbook of Toxic Fungal Metabolites. Academic Press, New York (in press).

Cole, R.J., Dorner, J.W., Cox, R.H. and Cunfer, B.: The isolation and identification of several trichothecene mycotoxins from Fusarium heterosporum. J. Nat. Prod. (in press).

Cole, R.J., Wells, J.M., Cox, R.H. and Cutler, H.G.: Deacetylcytochalasin H, a new [11] cytochalasin from Phomopis sp. J. Agric. Food Chem. 29, 205 (1981).

Cutler, H.G., Crumley, F.G., Springer, J.P. and Cox, R.H.: Dihydropergillin: A fungal metabolite with moderate plant growth inhibiting properties from Aspergillus ustus. J. Agric. Food Chem. (in press).

Springer, J.P., Cutler, H.G., Crumley, F.G., Cox, R.H., Davis, E.E. and Thean, J.E.: Plant growth regulatory effects and stereochemistry of cladosporin. J. Agric. Food Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30062-04 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Immunochemistry of Dioxin Action on the Lymphocyte and Other Halogenated Aromatic Hydrocarbons

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

PI:	M. I. Luster	Research Microbiologist	LEC NIEHS
OTHER:	G. A. Boorman	Research Pathologist	EBB NIEHS
	J. H. Dean	Research Microbiologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The effects of pre/postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on various immunological, bone marrow and host susceptibility assays were examined previously in $B_6C_3F_1$ hybrid mice. These studies were continued with several other compounds including a biphenyl (non-halogenated) chemical, orthophenylphenol. The effects of orthophenylphenol (OPP) and tris(2,3-dichloropropyl)phosphate (Fyrol FR2) on immunological functions and host susceptibility to infectious agents were examined following subchronic exposure to nontoxic levels in adult mice. Also included in these studies as a positive control were mice treated with cyclophosphamide (CY), a known immunosuppressant and cytoreductive drug. Exposure to relatively high doses of OPP (up to 200 mg/kg body weight per day for 10 days) failed to alter immune functions or host susceptibility. Fyrol FR2 treatment induced minimal changes in immune functions and host susceptibility only at the highest dose tested (25 mg/kg per day for 4 days) as indicated by decreased lymphoproliferative responses to mitogens and increased tumor takes following tumor cell challenge. In contrast, CY treatment resulted in marked alterations in both immunological functions and host susceptibility.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Alterations in bone marrow were determined by various *in vitro* and *in vivo* colony growth assays, bone marrow smears and quantitation of cellularity. Suppressor activity was examined by routine coculture experiments followed by determination of lymphoproliferative responses. Host susceptibility was examined by examining survival following injection of infectious agents on tumor development.

MAJOR FINDINGS AND PROPOSED COURSE: OPP is not immunotoxic indicating that the presence of halogens and their placement in the ring are critical for immunotoxicity. The observation that toxicity may occur without immunological affects provides further evidence that selected aromatic hydrocarbons have a selective effect on the immune system. The proposed course of studies regarding polyhalogenated aromatic hydrocarbons is uncertain and will depend upon availability of particular isomers.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Ingestion of many aromatic hydrocarbons appears to exert toxicologic effects in man and animals. Among these effects may be a modulation of the immune response, either suppression or enhancement. It is important to establish the nature and mode of action of this immune modulation in order to evaluate the possible hazards for man and animals with respect to its affect on host resistance to infectious agents and tumor development.

PUBLICATIONS

Luster, M.I., Boorman, G.A., Dean, J.H., Harris, M.W., Luebke, R.W., Thigpen, J.E., Padarathsingh, M.L. and Moore, J.A.: Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to TCDD. Int. J. Immunopharmacol. 2: 301-310, 1980.

Luster, M.I., Dean, J.H., Boorman, G.A., Archer, D.L., Lauer, L., Lawson, L.D., Moore, J.A. and Wilson, R.E.: The effects of orthophenylphenol, tris(2,3-dichloropropyl)phosphate, and cyclophosphamide on the immune system and host susceptibility of mice following subchronic exposure. Toxicol. Appl. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30063-04 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Orbital Calculations on Molecules of Environmental Interest

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

PI:	C. E. Parker	Research Chemist	LEC NIEHS
OTHER:	J. R. Hass	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

No work was performed during this reporting period due to lack of funds.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Programs were obtained from the Quantum Chemistry Program Exchange. These programs were modified as needed and run on the IBM 360/70 at the Triangle Universities Computation Center.

MAJOR FINDINGS AND PROPOSED COURSE: No work was performed during this reporting period as a result of funding priorities.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry is widely used for the identification and analysis of compounds of environmental interest. Mass spectrometry allows one to deduce structure through spectral interpretation, even where no reference compound is available. Correct interpretation of the data depends on the understanding of the gaseous ion chemistry involved. Molecular orbital theory allows the calculation of minimum energy configurations and charge distributions which are required for a complete understanding of fragmentation reaction pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30064-04 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Field Desorption Mass Spectrometry Studies

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

PI: J. R. Hass Research Chemist LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)
Dr. R. Teoule, Department of Radiobiology, Nuclear Research Center, Grenoble, France

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Protonated molecular ions were observed in the field desorption mass spectra of selected dinucleotides, cysteine and glutathione conjugates and porphyrins in addition to metal adduct ions. Technical progress was made in emitter preparation, mass calibration and general sample handling.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Field desorption mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 168 samples (62 as service work) were run by FD in 1980. The technique involved manual increases of emitter heating current while recording scanned mass spectra. This method is difficult to reproduce and is presently being replaced by electronic control of the emitter heating current.

The basic advances were technical in nature: 1) development of experience in growing consistent high-quality emitters; 2) development of reference compounds to allow generation of calibration tables from field-ionized materials; 3) development of technique in sample-handling (wire loading, addition of cationization reagents, etc.).

Promising results were obtained with a variety of polar compounds including dinucleotides, cysteine and glutathione conjugates and porphyrins. In general, protonated molecular ions and metal adduct ions are observed. Higher emitter currents often lead to more fragmentations, however.

Future developments include the electronic control of the emitter heating current and possibly the use of silicon-based emitters. A more recent development is the fast atom bombardment source. This technique, now commercially available, employs a beam of accelerated argon atoms to effect ionization of the sample. It is reported to have a much better success rate than FD, and to give much larger ion currents, which should improve sensitivity. Samples of polypeptides up to 2800 daltons have been reported to give molecular ions by this technique.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Finding mass spectrometric methods for the analysis of small polynucleotide-carcinogen adducts and other small molecule/biopolymer adducts will permit a rapid method for identification of these compounds, permitting studies of the effects of neighboring groups upon the reactivity of the site attacked. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30065-04 LEC

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of Mass Spectral Reactions in Field-Free Regions

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

PI:	J. R. Hass	Research Chemist	LEC NIEHS
OTHER:	L. A. Levy	Research Chemist	LEC NIEHS
	J. Yinon	Visiting Scientist	LEC NIEHS
	M.-J. Bobenreith	Visiting Fellow	LEC NIEHS
	T. A. Lehman	IGPA Visiting Scientist	LEC NIEHS

COOPERATING UNITS (if any)
M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC.

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A careful study of collision induced bond-breaking reactions in carbon cage compounds revealed that a substantial fraction of the fragmenting ion received 9-15 eV of internal energy during the collision process. Collision-induced-decomposition (CID) reactions were found to be useful for structural analysis of di-2-ethylhexylphthalate metabolites. A new tandem quadrupole mass spectrometer system has been developed for MS/MS studies. Kinetic energy release studies suggest the presummed retro-Diels Alder process in 5, 6, 6a, 7, 12, 12a-hexahydrobenzo[a]anthracene and related compounds is step-wise.

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: The loss of carbon containing fragments from cubane, mirex and kepone requires breaking a minimum of three carbon-carbon bonds. These bond strengths may be estimated from various heats of formation and ionization potentials. The observed spectra require a minimum of 9 eV of energy be transferred in the more energetic collisions.

Unimolecular and collision-induced decompositions (CID) of selected ions from the mass spectra of 5, 6, 6a, 7, 12, 12a-hexahydrobenzo[a]anthracene and related compounds could best be interpreted in terms of a step-wise rather than concerted process for the Diels Alder reaction. In particular, the kinetic energy release associated with the formation of $(M-C_8H_8)^+$ and $(M-C_8H_9)^+$ from the molecular ion was comparable for all compounds studied and was similar to the kinetic energy release for the $M^{+\cdot} \rightarrow C_8H_8^{+\cdot} + R$ transition. Since the loss of C_8H_9 is necessarily step-wise, it is implied the other two reactions are as well.

The methane positive chemical ionization mass spectra of di-2-ethylhexylphthalate metabolites give an ion (x^+) which contains the metabolized portion of the molecule. By subjecting this ion to CID-MIKES analysis, it was possible to distinguish the various metabolites.

Tandem quadrupole mass spectrometry has been proposed as a useful method of attacking a number of analytical problems. In order to evaluate this technique, a double quadrupole instrument similar to that described by Siegel (Anal. Chem. 52, 1790-1792, 1980) has been constructed. This instrument has been successfully operated in the electron-impact mode using direct probe or capillary column gas chromatography for sample introduction. Chemical ionization results have been erratic and limited success with direct coupled high pressure liquid chromatography has been achieved. A comparison of quadrupole MS/MS with CID-MIKES revealed the latter gave more extensive fragmentation but little additional specific information.

The major emphasis of this project will be the coupling of chromatography with either MS/MS or CID-MIKES. The acquisition of the fast atom bombardment source opens such potential applications as direct-coupled thin-layer chromatography/MS/MS.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry (in combination with chromatography) is the most sensitive and specific analytical method presently available for dealing with complex samples of environmental/biological origins. The results of this project will increase our understanding of the fundamentals of the technique as well as provide more specific information for qualitative or quantitative analysis.

PUBLICATIONS

Harvan, D.J., Hass, J.R., Friesen, M.D. and Albro, P.W.: The mass spectrometry of metabolites of di-2-ethylhexylphthalate. Biomed. Mass Spectrom. 7, 242-246 (1980).

Lehman, T.A., Harvan, D.J. and Hass, J.R.: Bond breaking energy in collision-induced-dissociation: A study of carbon cage fragmentation. Org. Mass Spectrom. 15, 437-439 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30066-04 LEC

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Synthetic Methods for Polyhalogenated Aromatics

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

PI: J.D. McKinney Supervisory Research Chemist LEC NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Synthesis and characterization of brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs continues. These include pentabromomethylnaphthalene, 14-labeled 1,2,4,6,7-pentabromonaphthalene and work toward modification of the 1,2,4,6,7-pentabromonaphthalene as a haptenic compound in radioimmunoassay work.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: Efforts are continuing on the synthesis and characterization of brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs. A pentabromomethylnaphthalene has been synthesized from direct bromination of 2-methylnaphthalene and determination of the exact structure is in progress. ^{14}C-Labeled 1,2,4,6,7-pentabromonaphthalene has been synthesized from the direct bromination of ^{14}C-labeled naphthalene. The 1,2,4,6,7-pentabromonaphthalene is also being synthetically modified to provide a haptenic compound for radioimmunoassay purposes.

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.

FLORIDA STATE UNIVERSITY - Tallahassee, Florida 32306
(NIH-ES-79-2)

TITLE: Rapid Screening Analysis for Aromatic Hydrocarbon Residues

CONTRACTOR'S PROJECT DIRECTOR: R.C. Dougherty, Ph.D.

PROJECT OFFICER (NIEHS): J.R. Hass, Ph.D., Research Chemist, LEC

DATE CONTRACT INITIATED: March 21, 1979

CURRENT ANNUAL LEVEL: $79,000

PROJECT DESCRIPTION

OBJECTIVES: To develop validated methods for screening biological/environmental samples for aromatic hydrocarbons and provide increased capacity for the analysis of "targeted" samples.

METHODS EMPLOYED: Solvent extractions, chromatography, negative chemical ionization mass spectrometry, electron capture gas chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor developed a method for the analysis of gauze pads suitable for use in swipe tests. After he was confident his method was performing properly, he was sent spiked samples and blanks. It was impossible to distinguish between the spikes and blanks, based upon his results. The contractor reported analysis for polychlorinated organic compounds in human milk from mothers in Tallahassee, Florida and Hunterdon, New Jersey and infant formula preparations. It was determined that lipid matrices can have a very significant effect upon evaporation profiles in direct probe NCI screening. The contractor proposed structures for several polychlorinated organic compounds that have turned up in human biofluids.

Due to change in priorities at the Institute and the disappointing results of the blind sample studies, this contract has been terminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The rapid reliable identification of possible toxicants involved in environmental exposures is essential for public health officials to react in the most appropriate manner. This contract enabled us to evaluate the reliability of methods which are being actively promoted for general usage.

RALTECH SCIENTIFIC SERVICES - Madison, Wisconsin 60616
(NIH-NO1-ES-7-2141)

TITLE: Analysis of PCB's and DDE in Human Body Fluids and Tissue

CONTRACTOR'S PROJECT DIRECTOR: D.L. Hughes

PROJECT OFFICER (NIEHS): J.D. McKinney, Ph.D., Chief, LEC
D.B. Walters, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: September 30, 1977

CURRENT LEVEL (1 year): $199,000

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, as well as a perchlorination technique.

MAJOR FINDINGS AND PROPOSED COURSE: This contract provides for development of the methodology for the analysis and the generation of suitable and reliable analytical data on PCB's and DDE in human body fluids and tissues and is interfaced with contract (NIH-NO1-ES-8-2105) for the analysis of total organic chlorine and bromine in same media. Method validation and recovery studies for human milk, blood (serum) and placental tissue have been completed. Protocols for the analyses have been developed and approved for use. The contractor has set up a quality control program and analysis of the mother's samples is in progress. The contractor is serving as sample split point for the study. They will do first thaw and homogenization of sample, remove aliquots for other contractor and our purposes and retain and weigh in sample for their purposes. Several hundred mother's samples have been received by the contractor.

This is the final year of the contract. There are 1200 samples yet to be analyzed to give a total of 4000 samples. In addition, the PCB content of ten percent of this total number of samples will be confirmed by a perchlorination technique. Milk extract samples from each individual mother will be pooled, perchlorinated and analyzed for decachlorobiphenyl by EC-GC.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possible effect of transplacental and breast milk transfer of environmental contaminants from mothers to babies is an important and recurring epidemiological question. It has been shown that environmental contamination of breast milk occurs in the United States. Organohalogen pesticides and the polychlorinated biphenyls (PCB's) are widespread contaminants of breast milk. This study provides an integral part of an overall study of the possible widespread contamination of breast milk by environmental contaminants as PCB's and their effects on infant development and health.

SCIENCE APPLICATIONS, INC. - La Jolla, California 92038
(NIH-NO1-ES-8-2105)

TITLE: Analysis of Total Organic Chlorine and Bromine Residues in Human Body Fluids and Tissues

CONTRACTOR'S PROJECT DIRECTOR: J. Reed

PROJECT OFFICER (NIEHS): J.D. McKinney, Chief, LEC

DATE CONTRACT INITIATED: December 20, 1977

CURRENT LEVEL (2 years): $325,000

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: A new method based on Bio-Gel P-2 filtration has been developed to facilitate desalting of the human milk and serum samples. This method is compatible with NAA measurements. Additional methodology has been developed for milk substitutes (formula) and placenta tissue extracts. Analyses have been performed on about 1500 samples to date. Additional quality control experiments on the method are being performed as further validation.

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 inorganic 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.

SRI INTERNATIONAL - Menlo Park, California
(NIH-NO1-ES-79-0006)

TITLE: Application and Development of Procedures for the Analytical Determination of Environmental Chemicals by Radioimmunoassay

CONTRACTOR'S PROJECT DIRECTOR: Jack H. Pincus, Ph.D.

PROJECT OFFICER (NIEHS): Phillip W. Albro, Ph.D., Research Chemist, LEC

DATE CONTRACT INITIATED: June 1, 1979

CURRENT ANNUAL LEVEL: $174,089

PROJECT DESCRIPTION

OBJECTIVES: (1) To evaluate the performance of radioimmunoassays developed at NIEHS; (2) to develop suitable procedures for the application of the specified assays to environmental samples; (3) to apply the specified immunoassays to the analysis of samples.

METHODS EMPLOYED: Double-antibody radioimmunoassay, organic solvent extractions, chromatographic cleanup procedures, statistical analysis of data.

MAJOR FINDINGS AND PROPOSED COURSE: The initial report after 6 months of effort indicates that SRI has been able to perform the radioimmunoassay for chlorinated dibenzo-p-dioxins developed at NIEHS, and has confirmed NIEHS's findings relative to sensitivity and specificity of the assay. SRI has successfully produced antisera to the dioxin antigen in a large number of rabbits locally.

Through screening a large number of rabbits, antisera have been obtained that show greater specificity (less cross reactivity) than were available previously. Genetic variables seem to dominate the specificity of different antisera. Soil has proven a difficult matrix for this assay, but effective cleanup procedures are nearly through being developed.

We propose to (1) extend the validation studies to radioimmunoassays for polychlorinated biphenyls and dibenzofurans; (2) begin to analyze samples of environmental interest; (3) develop standardized workup procedures for soil, adipose and other appropriate matrices; (4) attempt to develop a solid-state assay technique.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One major purpose in developing these assays is the enabling of small, clinical laboratories to analyze samples for trace levels of these halogenated aromatic compounds without a need for extremely expensive, highly sophisticated instrumentation. An essential preliminary step is the demonstration that the NIEHS-developed techniques can be reproduced in other laboratories.

LABORATORY OF MOLECULAR GENETICS

THE LABORATORY OF MOLECULAR GENETICS

Summary Statement

The Laboratory of Molecular Genetics (LMG) was established to investigate basic molecular mechanisms of mutagenesis, to optimize mutagen test systems and to test large numbers of chemicals for mutagenic properties. Although quite different, these three programs are complementary and benefit mutually from their close association.

Both genetic and biochemical approaches are employed to develop an understanding of molecular mechanisms of mutagenesis. These basic studies bear directly on both the testing and test development programs of the LMG. An understanding of mutagenesis provides the framework from which new mutagen screening tests can be developed. Conversely, the identification of new chemical mutagens can provide insights into the mechanisms by which mutation occurs.

A large number of compounds, both natural and synthetic, have been identified as mutagens in a variety of test systems. The risk they may pose to human populations can by fully assessed only when the underlying mutagenic mechanisms are understood. Such an understanding demands detailed knowledge of specific targets within DNA, of the lesions formed and of the end products from the interactions among specific mutagens, their substrates and the enzymes of DNA metabolism.

THE MOLECULAR GENETICS PROGRAM

The group led by Dr. Akio Sugino is studying the replication of DNA, the basic determinant of heredity, in both prokaryotic and eukaryotic systems. The prokaryotic systems focus on the first steps of DNA synthesis, examining initiation of chromosomal DNA replication using a bacteriophage N4 *in vitro* DNA synthesis assay developed in his laboratory. The enzymology of Okazaki fragment priming and subsequent primer removal is being studied using a newly characterized mutant of *E. coli* deficient in RNaseH activity, a key enzyme in this process. Since the mechanism of DNA replication in higher organisms is poorly understood, much of the attention of this group is directed towards elucidating its biochemistry. The yeast 2 μm plasmid DNA is being used as substrate in an *in vitro* DNA replication system. DNA synthesis of this plasmid depends upon the same polymerase used to replicate chromosomal DNA, and has been shown to use the same replication origin *in vitro* and *in vivo*. Thus, this plasmid provides a useful tool for the isolation of enzymes and cofactors required for yeast DNA replication. Mutants of nuclear replication enzymes have been isolated in both yeast and *Drosophila*. These include the first unambiguous mutant eukaryotic polymerases to be discovered. The subset of mutants whose apidicolin resistance results from an altered polymerase enzyme are now being used to clone the yeast DNA polymerase gene.

The group led by Dr. Lynn Ripley concentrates on mechanisms of mutagenesis using the T4 bacteriophage system. This system has been used extensively over a considerable period of time and has provided much of the information now available concerning mutagenic processes. Projects currently underway have yielded new insights into mechanisms of frameshift mutagenesis, invoking the involvement of DNA secondary structure in the mispaired intermediates. The molecular actions of several chemical and physical mutagens are also being studied. Hydroxylamine (HA)

produces a number of adducts of cytosine. Using the T4 system and various HA derivatives, ratios of HA-induced adducts can be altered in order to assess their individual mutagenic potentials. Experiments supplying N^4OH cytidine to T4-infected E. coli have shown that previous results of other laboratories, suggesting that this particular HA-induced cytosine adduct produces only AT-to-GC mutations, were the result of inadequate detection of GC-to-AT mutations. The physical mutagen, heat, appears to act by inducing GC-to-AT transitions, and also transversion at GC sites.

The group led by Dr. David Mace studies the chemistry of mutagenesis, focusing on the intermediate steps between mutagenic treatments and alterations of DNA sequence. Their current studies focus on the chemical changes which occur during heat mutagenesis. The significant reaction during heat-induced depurination is postulated to be rearrangement of guanine nucleotides to form deoxyriboneoguanylic acid. This product is expected to be capable of base pairing with G instead of C. Preliminary evidence shows that neoguanosine can form in DNA polymers. Current experiments are designed to establish more conclusively its formation in DNA polymers and to determine its pairing properties in vitro.

Dr. Mark Conkling's research focused on two areas of mutagenesis: the effects of neighboring and nearby bases on site-specific mutagenesis, and the bacteriophage T4 misrepair system. The alteration of a specific base pair some 20 to 80 base pairs away from a mutational target site produces a marked effect on the frequency of both spontaneous and 2-aminopurine-induced reversion at the target site. While it has been known for some time that nearest neighbors can affect the mutation rate at a specific site, more distant neighbors have not previously been known to have such effects. The T4 misrepair system is encoded by the w, x, and y genes. Mutations in x and y (but not w) were shown to suppress mutations in gene 49. Using this property, new mutant alleles of x and y were isolated, including temperature-sensitive and amber alleles. Such alleles are especially useful for identifying the corresponding proteins and establishing their functions in the misrepair process.

Dr. John Drake has returned to the laboratory bench for short periods of time between administrative duties. Currently his investigations are aimed at establishing the mutagenic effects of bisulfite and determining the mechanism of methylmethanesufonate (MMS) mutagenesis in bacteriophage T4. Bisulfite is commonly used as a food preservative. A previous claim that bisulfite is mutagenic to T4 is suspect, since T4 contains hydroxymethylcytosine (HMC) in place of cytosine in its DNA and HMC is much less reactive with bisulfite than is cytosine. Experiments employing T4 mutants which contain cytosine rather than HMC in their DNA are planned to determine whether bisulfite is mutagenic with this type of substrate. Studies on the alkylating agent MMS have shown that two mechanisms are involved: one operates via the error-prone DNA repair pathway, while another is independent of this system.

The group led by Dr. Michael Volkert investigates the impact of DNA repair mechanisms on the mutagenic process. Studies have focused on the role of the recF gene in the regulation of the E. coli inducible DNA repair system. This repair system is expressed only after DNA damage, for instance UV irradiation. Its full expression requires the activity of several gene products including recF itself. Current results suggest that recF may be needed in the early steps of the regulatory process. In addition, its activity may be required more directly for

the expression of induced mutagenesis. Another study nearing completion concerns the conservation of damaged DNA. In order for normal levels of DNA repair to occur, the DNA substrate for recombinational repair must be protected from exonucleases by the host's inducible DNA protection mechanism. The nature of this protection is under investigation using mutants which protect DNA but do not show the usual correlation with specific protein expression. This suggests either that an alternative DNA protection mechanism exists or that current hypotheses regarding the nature of this protective mechanism are incorrect. A study of DNA repair mechanisms acting on alkylation damage has also been initiated, concentrating on the isolation of E. coli mutants defective in such repair.

THE MUTAGEN TESTING PROGRAM

This program is designed to test chemicals for potential mutagenic properties and to optimize mutagen tests. The testing is done via contracts which are developed and supervised by LMG personnel. In addition, these scientists also head research groups conducting investigations into the mechanisms behind the mutagen screening tests, the development of new tests and improvements upon existing tests.

The group led by Dr. Errol Zeiger concentrates on the Salmonella reversion test system for mutagen screening. Constancy of the genotype of the strains used in testing is a crucial requirement for reproducibility and reliability. Two projects are in progress to prevent mutagen testing complications arising from the relative instability of bacterial strains used in these tests. The first evaluates the degree of instability of the several tester strains by periodic evaluation of their genotype during long-term storage. The second involves the development of a simple, rapid test to confirm the phenotype of all tester strains used in this screening program; this is achieved by monitoring the differential responses of each strain to several mutagens, and can be performed concurrently with the mutagen test. Many of the compounds examined in the Salmonella test are mutagenic only after metabolic activation by liver or lung "S9" extracts. Differential activation of specific compounds by extracts of liver and lung has been linked to different levels of cytochrome P-450 isozymes present in these two tissues. The lung contains more of the $P\text{-}450_I$ isozyme than does the liver, resulting in greater activation and higher levels of mutagenic activity for 2-aminoantracine, 2-aminofluorine, and 2-acetylaminofluorine. Host-mediated assays to determine organ specificity of mutagen activation are also being developed to better understand the metabolic activation process.

The group led by Dr. James Mason studies mutagen-sensitive mutants of Drosophila. Characterization of the mei-41 and mus-104 mutants suggests that they are allelic but have different effects on DNA metabolism. Both mutations cause methylmethanesulfonate (MMS) sensitivity, but only mei-41 affects meiosis. This implies different active sites within the gene, and suggests that different activities are involved in resistance to MMS and meiosis. The linkage of DNA repair of genetic damage to mutation is being established, as is the identification of the genes controlling these processes.

The group led by Dr. Michael Resnick is studying DNA repair in yeast cells. This model eukaryotic system has the advantage of a large number of well characterized mutants deficient in various types of DNA repair enzymes. The analysis involves

the isolation of chromosome-sized pieces of DNA and their sedimentation on sucrose gradients to determine changes in the structure of DNA during the repair process. It has been established that several of the DNA repair processes are involved in meiosis and that excision repair in mitotic cells shares genetic and physiological properties with that in meiotic cells. The rad52 gene, which is required for x-ray damage repair, is also involved in the meiotic process: meiosis results in much lethality in rad52 mutants, presumably due to the accumulation of single-strand DNA breaks during this process. (These single-strand breaks may initiate recombination events which require rad52 for resolution.) These results link the mechanism of DNA repair to a specific mechanism in the metabolism of undamaged DNA occuring during meiosis. In order to facilitate the more efficient handling of the vast amounts of data accumulated during the analysis of sucrose gradients, an interactive computer program has been developed to store and analyze data received directly from the scintillation counter. This program allows the rapid determination of molecular weights of DNA from gradient profiles.

ENVIRONMENTAL MUTAGEN TEST DEVELOPMENT PROGRAM (EMTDP)

The chemical testing program of the LMG, EMTDP, is designed first to test chemicals for mutagenicity and second to evaluate the tests themselves. To meet these two aims, contracts are let with several agencies and organizations to test both chemicals of unknown mutagenicity and, in parallel blind studies, control chemicals whose mutagenic properties are known. In this manner laboratory-to-laboratory variation can be examined, test standization procedures established and tested, and the potential mutagenicity of new chemicals reliably determined. The contract process is also used to test chemicals for genetic effects other than mutagenicity. (Such effects cannot be determined in the bacterial tests.) The ability of chemicals to induce chromosomal aberations in cultured chinese hamster ovary cells are being tested at two institutions. Aneuploidy is another type of gross genetic change which can occur as a result of exposure to environmental hazards. Two new tests using yeast and Drosophila test systems are currently being developed to screen chemicals for potential to cause this type of genetic change. These tests will greatly enlarge the scope of the screening which can be done to determine the amount of risk that is posed to the genetic material by environmental chemicals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60052-05 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Environmental Mutagenesis Test Development Program

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG	NIEHS
Other:	B. Margolin	Mathematical Statistician	BB	NIEHS
	J. Mason	Research Geneticist	LMG	NIEHS
	M. Resnick	Research Geneticist	LMG	NIEHS
	M. Rowley	Computer Systems Analyst	BB	NIEHS
	D. Walters	Chemist	NTP	NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objectives of the Environmental Mutagenesis Test Development Program are twofold. The first is the development of an integrated program for the routine testing of large numbers of chemicals for mutagenicity. This includes such diverse factors as test-system validation, identification and development of new test methods, development of an increased understanding of mutagenesis and development of a strategy and an organizational structure for conducting a large-scale testing program and interpreting the results obtained. The second objective is implementation: to test large numbers of commercial and environmental chemicals each year for mutagenicity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Mutagenicity testing: Testing is underway in three contract laboratories using the Salmonella test system of Ames. These laboratories are: Case Western Reserve University, Cleveland, Ohio; EG&G Mason Research Institute, Rockville, Maryland; and SRI International, Menlo Park, California. These laboratories have tested a total of 363 samples to date. Also underway are three contract laboratories using Drosophila melanogaster. These laboratories are: Bowling Green State University, Bowling Green, Ohio; Brown University, Providence, Rhode Island and University of Wisconsin, Madison, Wisconsin. These laboratories have tested a total of 20 coded chemicals to date.

Test System Development and Validation: Protocols have been developed in two contract laboratories for cytogenetic and sister chromatid exchange evaluation in Chinese hamster ovary cells in culture. These laboratories are: Columbia University, New York City, New York, and Litton Bionetics, Inc. Rockville, Maryland. These laboratories have standardized and validated a protocol using 15 open and coded chemicals. This protocol includes a methodology for selecting dose levels at which to test. The standardized protocol produced results that were reproducible across laboratories. The contractors have just begun to test coded chemicals using this newly developed and validated protocol. A contract has been awarded to Brown University, Providence, R.I., to develop a protocol that will be usable for the detection of aneuploidy and to use that protocol to test a number of chemicals. The protocol development effort has just commenced.

Chemicals: A contract is ongoing at Radian Corporation, Austin, Texas, to maintain a chemical respoitory and analytical facility for the Environmental Mutagenesis Test Development Program. The repository is shipping coded chemicals to all contractors for mutagenicity testing. In addition they have begun analysis of chemicals found to give either positive or questionable responses in Salmonella. A number of chemicals have been transferred from the NCI repository to the EMTDP repository for testing in Salmonella. This contract will terminate this year so is being advertised for competitive award.

Data Handling: An advisory group to the EMTDP has recommended that EMTDP use the PROPHET computer system developed by Bolt Beranek and Newman, Inc., Cambridge, Massachusetts under contract to Division of Research Resources, NIH as a data base management system. The system will enable the Project Officer at NIEHS to reconstruct the experiment, retrieve data, monitor levels of effort and quality of the work, summarize data within and across laboratories, perform statistical operations on the data, and produce management reports.

FUTURE CONTRACTS: Contracts will be awarded this year to study the induction of nondisjunction, to be measured as aneuploidy, in yeast. The purpose of these contracts would be: 1) to develop a protocol that is usable for detection of aneuploidy and which is applicable for large-scale testing of substances; 2) to validate the protocol with a number of uncoded and coded substances.

Additional contracts will be awarded to develop and validate a multiple endpoint mutation system in cultured mammalian cells and to develop modified Salmonella test protocol(s) for detection of chemicals requiring reductive/anaerobic metabolism for their mutagenic activity. The protocols developed in both contracts will be used to test a series of coded chemicals for mutagenicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
There are thousands of chemicals in use and the number is ever increasing. The majority of these substances have not been evaluated for the potential to induce heritable genetic alterations; and, hence, the possible risk to future human generations is unknown. This program will develop an efficient, coordinated system for mutagenesis testing and mutagenesis data management and should serve as a model system. The data obtained from this program will be useful for decision making on the safety of various classes of chemicals to which man is exposed.

PUBLICATIONS

Zeiger, E. and J. W. Drake, (1980). An Environmental Mutagenesis Test Development Program, in Molecular and Cellular Aspects of Carcinogen Screening Tests. Ed. R. Montesano, H. Bartsch and L. Tomatis. IARC Scientific Publications No. 27., Lyon, p. 303-313.

Zeiger, E. (1980). The National Institute of Environmental Health Sciences mutagen testing program. Mutation Res. 74: 170.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60077-04 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Heat-Induced Mutation

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

PI: L. S. Ripley Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Heat-induced mutations in bacteriophage T4 have been demonstrated to occur by at least two distinct mechanisms: (1) G·C base-pairs are converted to A·T base-pairs (transversion). The C of the base-pair is the target of the first mechanism. The G of the base-pair is the target of the second mechanism. Studies of the step(s) required for conversion of the heat-induced lesion (pre-mutational lesion) to the fully expressed mutation are continuing. The specificity of the transversion reaction (G·C to T·A versus G·C to C·G) is to be determined. T4 phage contain glucosylated hydroxymethylcytosine in their DNA. The influence of this modification on mechanism (1) will be explored.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 are used. Mutations are measured in the rII genes. Appropriate mutants are available for making T4 with DNA which is non-glucosylated or which has C rather than hydroxymethyl-C.

MAJOR FINDINGS AND PROPOSED COURSE: In tests of the mispairing properties of heat-induced DNA lesions made by measuring the number of mutant progeny descendents from parental phage treated with heat, transition mutations (G·C → A·T) were found to be produced with high probabilities per round of replication. In contrast, mutations yielding transversion mutations at G·C sites were found to produce mutations with very low probabilities per round of replication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This type of characterization of the mutagenic outcome of mutational lesions is valuable in setting limits for the quantity of a lesion which must be present in order to account for the mutagenic outcome and thus finally helpful in identifying the potential DNA lesion induced by heat which are responsible for mutation.

PUBLICATIONS

Ripley, L. S., Heat mutagenesis in bacteriophage T4: A quantitative characterization of mutation production for heat-induced DNA lesions, in preparation.

Ripley, L. S., Heat mutagenesis in bacteriophage T4: Genetic properties of heat-induced lesions producing transition mutations, in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60080-04 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Modification of Site-Specific Mutation Rates by a Nearby Base-Pair Substitution

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

PI: M. A. Conkling Geneticist LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Some evidence supports the hypothesis that the mutation rate at a particular site is influenced by neighboring base-pairs. I have sought to modify the reversion and conversion frequencies of bacteriophage T4 rII nonsense codons by inserting nearby temperature-sensitive rII lesions. The insertion of a ts mutation reduces the 2-aminopurine-induced reversion of an rII amber mutation about three-fold. The ts mutation reduces the corresponding UAA-UGA conversion about eight-fold, while the reversion of the ochre codon to glutamine (UAA→CAA) is not affected. Selection controls show no measurable selection against the ts marker. DNA sequence analysis suggests that the rate-modulation effect of the ts mutation may occur because of a change in DNA secondary structure.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Double rII mutants have been constructed composed of one of two homologous nonsense mutants (rHB129am and rHB129oc) plus a ts mutation (rPS78). The spontaneous and 2-aminopurine-induced reversion and conversion frequencies of the nonsense mutations are measured in the presence or absence of the ts mutation.

MAJOR FINDINGS AND PROPOSED COURSE: The spontaneous and 2-aminopurine-induced reversion frequencies of rHB129am are reduced about threefold in the presence of rPS78. The modified reversion frequency does not appear to reflect an altered efficiency of detection of revertants, but rather an alteration in the mutation rate itself. The 2-aminopurine-induced reversion of rHB129oc is not affected by the ts mutation. The 2-aminopurine-induced conversion of rHB129oc to rHB129am is reduced approximately three-fold in the presence of the ts mutation. The 2-aminopurine-induced conversion of rHB129oc to rHB129op is reduced about eight-fold in the presence of the ts mutation. Genetic evidence suggest that about 30 base-pairs separate the rHB129 site from the rPS78 site. The precise physical distance will be determined by molecular cloning and DNA sequencing of this region of the T4 rIIA gene.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The characteristic mutation rate of a genetic site is often supposed to depend not only upon the base-pair at that site, but also upon the molecular environment determined by nearby base-pairs. This idea seems to have originated as an explanation of mutation "hot spots". A rigorous demonstration of a neighboring base effect requires that a mutation rate be altered as a result of a nearby base-pair substitution. An understanding of the fundamental mechanisms of mutagenesis requires an understanding of the role of the molecular environment upon mutation.

PUBLICATIONS

Conkling, M. A., Koch, R. E., and Drake, J. W.: Determination of mutation rates in bacteriophage T4 by unneighborly base pairs: genetic analysis. J. Mol. Biol. 143: 303-315, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60081-04 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Genetics of the Misrepair Mutagenesis System of Bacteriophage T4

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

PI: M. A. Conkling Geneticist LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Lesions induced in DNA may be repaired by "error-proof" or "error-prone" repair mechanisms. Error-prone systems repair the lethal damage of the lesion, but in doing so may fail to restore the original nucleotide sequence, producing mutations. This error-prone process is called misrepair mutagenesis. Bacteriophage T4 has its own misrepair pathway, mediated by at least three genes, x, y and w. Mutations in two of these genes, x and y, suppress mutations of gene 49, a gene involved in DNA packaging. This allows the selection of alleles of x and y; ts alleles of x and y have been selected. These are temperature sensitive both for suppression of gene 49 amber mutants and for UV sensitivity. Amber alleles of x and y are being sought. Using these and other x and y alleles, the genes x and y will be mapped. The ts alleles will be used to study the time course of mutagenesis in T4.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Suppressors of gene 49 amber mutations will be selected. These can be arranged into complementation groups using a complementation test based on UV sensitivity. Their sensitivities to inactivation and mutagenesis by UV irradiation will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: Mutations in genes involved in T4 misrepair mutagenesis result in decreased recombination frequencies, increased sensitivity to inactivation by UV or gamma irradiation, and decreased sensitivity to mutagenesis by UV or gamma irradiation. Mutations in genes x and y can suppress amber and ts mutations of gene 49, a gene involved in DNA packaging. ts alleles of x and y have been isolated. These are ts both for suppression of gene 49 amber mutations and for sensitivity to inactivation by UV irradiation. Preliminary evidence suggest that the suppression of the gene 49 defect is an early function. This might provide an assay for the x and y gene products in vitro. Amber alleles of x and y are being sought. With these, the x and y gene products might be visualized on two-dimensional gels. Temperature shift experiments using the ts alleles will allow a study of the time-course of mutagenesis. The genes x and y will be mapped using alleles selected by their ability to suppress gene 49 mutants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the misrepair pathway is thought to be a major pathway of mutagenesis in eucaryotic systems, the biochemistry of this repair process is not understood. Bacteriophage T4 offers a simple model system to begin to understand the enzymology of the misrepair process and also offers a highly defined genetic system to study mutagenesis along certain defined pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60082-04 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Spontaneous Changes in DNA Primary Structure

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

PI:	D. C. Mace	Senior Staff Fellow	LMG	NIEHS
Other:	B. D. Price	Biologist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project investigates the possibility that deoxyneoguanosine residues in DNA (hyprothesized to be the G·C → C·G transverison pathway intermediate for heat mutagenesis in bacteriophage T4 can form a base pair with a deoxyguanosine residue on the opposing strand of a double helix. The formation of this novel base in polymeric and monomeric form is being studied. Its capacity to form a base pair with guanosine residues will be approached by both physical and biochemical methods.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Column chromatographic and thin layer chromatographic schemes have been worked out for efficient separation of neoguanylic acid from guanylic acid as well as from a variety of other heat products formed under certain conditions. These methods have been employed to isolate modest amounts of the desired product as well as to study the rate and mechanism of its formation under various experimental conditions. Established techniques are being used to convert the monophosphate to the di- and triphosphates. A direct chemical synthesis of neoguanosine is being attempted and if successful will allow more convenient preparation of large amounts of the compound. Detection of neoguanosine in DNA at this time depends on radioactive labeling of the initial material, enzymatic degradation and thin layer separation of the resultant products. Alternate approaches to be attempted include preparation of specific antibody to neoguanosine from rabbits injected with neoguanosine chemically coupled to protein carrier molecules. Finally, direct chemical synthesis will allow synthesis of heavy-isotope-labeled neoguanosine and open the possibility of mass spectral analysis of deoxyguanylic acid in natural DNA's.

MAJOR FINDINGS AND PROPOSED COURSE: To date we have found that during the early stages of depurination of these compounds there is one principle side reaction. This constitutes an apparent migration of the sugar attachment to the base from the normal N_9 endocyclic nitrogen of guanine to its exocyclic amino group. The product, N_2 ribosyl guanine, was identified some years ago as a minor contaminant of commercial GMP preparations and given the name neoguanylic acid. This compound, both in its ribo- and deoxyribo forms has been characterized chemically and biochemically and isolated in modest amounts.

Small amounts of the triphosphate of deoxyriboneoguanylic acid have been synthesized with the intent to obtain DNA polymers containing this residue. Unfortunately, this aspect of the project has not yet proved successful, but efforts in this direction are continuing. The diphosphate of riboneoguanylic acid is expected to be prepared at the end of the summer in order to synthesize ribopolymers containing this residue. If these polymers are obtained, we will use them to investigate the base pairing properties of neoguanylic acid with other residues using classical polymer mixing techniques.

Work currently in progress on the rearrangement of guanine nucleotides is focusing on two aspects of the problem. Initial studies with DNA labeled in the dGMP residues showed the time and temperature dependent appearance of radio-activity co-chromatographing with authentic deoxyneoguanylic acid. We are now attempting to show clearly that deoxyneoguanylic acid is in fact formed in DNA. This is being done by working with non-radiactive DNA samples on a scale large enough to permit spectroscopic characterization of any rearrangement products of the dGMP (and perhaps other DNA constituent) residues. If we can show that at least the UV spectrum of the product is correct, we can then feel confident that the product that we have identified radiochemically is in fact deoxyneoguanylic acid.

In addition to substantiating the formation of deoxyneoguanylic acid in DNA, we are also pursuing studies on the mechanism of formation of (deoxy) neoguanylic acid. Using free base and ribose-5'-phosphate, we have shown that neoguanylic acid can be formed directly in aqueous. Thus during depurination the free base and sugar phosphate that are formed can react to yield neoguanylic acid. Of course this mechanism can be reasonable for DNA as well, at least up to when the free base generated by depurination difuses out of the DNA helix.

During the course of these mechanists studies, we have also observed that the reaction of exocyclic amino groups with ribose-5'-phosphate appears to be a general one. A number of amino-purines and pyrimidines have been or are being tested in this reaction. Of most interest at the moment, adenine appears to react almost as well as guanine, yielding "neoadenylic acid". Whether or not the reaction can also occur at the endocyclic nitrogens is still an open question. This will be persued once the basic work on neoguanylic acid is complete. However, it can be noted that there are products, which are formed concomitantly with neoguanylic acid, that are clearly some type of sugar phosphate attached to a guanine.

Finally, based on these studies with the direct reaction of the examine group of a free base with a sugar phosphate, we will immediately proceed to a synthesis of these compounds. This should be possible using non-aqueous solvents and activating the C-1 position of the sugar by the classical means of synthesizing a C-1 halo sugar.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The heat-induced G·C → C·G pathway could be a major contributor to the mutational load sustained by the mammalian genome. Thus, it seems important to determine if the hypothesized transversion pathway (involving a thermally induced migration of the glycosylic bond of deoxyguanosine) actually does occur and is able to form a base-pair with guanosine during the synthesis of DNA by T4 DNA polymerase. If the results indicate that deoxyneoguanosine is involved, investigations into the existence of this pathway and its repair will be pursued in mammalian cell systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60101-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of a Computerized Data Base Management System for the EMTDP

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG	NIEHS
Other:	M. Rowley	Computer Systems Analyst	BB	NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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 (200 words or less - underline keywords)

The Environmental Mutagenesis Test Development Program (EMTDP) will be generating large volumes of data and experimental information on all chemicals which will be tested for mutagenicity. A computerized data base management system will be required to capture this information in an interactive mode in the testing laboratory, store, process, and analyze the data, and provide summary analyses to the experimenter and to the EMTDP project officers. This system will also allow the EMTDP staff to follow the course of testing with time in the large number of laboratories regardless of the mutagenicity test system being used. The PROPHET system developed and managed under contract to NIH/DRR, has been selected. It is being adapted to serve as the EMTDP data base management system.

PROJECT DESCRIPTION

METHODS EMPLOYED: An ad hoc advisory group was formed to aid the EMTDP and Biometry Branch staffs in defining computer needs and the identifying of an acceptable system.

MAJOR FINDINGS AND PROPOSED COURSE: A system survey identified one system, PROPHET, developed for NIH DRR by Bolt, Beranek and Newman (BBN) which would meet the needs of the EMTDP.

Work has been completed at BBN on a laboratory data entry terminal to be used for EMTDP data collection and on the data base structure and software required to store this data on PROPHET. This data entry terminal is in place in the Salmonella testing laboratories and is being used to transmit data directly to PROPHET thereby obviating the need for data forms. Once in PROPHET the data is checked for completeness and made available to the EMTDP project officer in an easily readable form for approval. After approval, the data is stored in readily accessable tables for future reference and summary management tables are updated with the new information. Data from other test systems is being entered into PROPHET by NIEHS personnel.

Analytical techniques required to perform mutagenicity and quality control determinations are being developed by EMTDP scientists for addition by BBN into PROPHET. Finally, BBN is currently working on new interactive and batch report generation routines to facilitate the management of the EMTDP results.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This system will allow the timely and accurate collection and retrieval of laboratory test data, and will also provide EMTDP management with a tool to manage the results, produce management reports and provide analyses to back-up mutagenicity determinations. It is the first system of its type to provide all of these facilities in such a fashion for use by non-computer-oriented management personnel.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60102-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Testing of Chemicals of Interest in Salmonella

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG NIEHS
	D. Pagano	Research Microbiologist	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 are being used to test chemicals of interest for mutagenicity. A series of cyclic hydrazides which are analogs of nitrosamines have been tested for mutagenicity for comparison with mutagenic and non-mutagenic nitrosamine counterparts. In addition Dimethylaminoazobenzene (Butter yellow) and two of its analogues, as well as 2,3,7,8-Tetrachlorodibenzofuran and alkylnitrites will be tested.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test procedure of Ames with some modification or liquid suspension tests were used.

MAJOR FINDINGS AND PROPOSED COURSE: All hydrazides were mutagenic for TA-1535 and TA-100 in the absence of S9. No activity was seen against TA-98 either with or without S-9. TA-1535, in all cases, showed a consistently higher response than TA-100. The mutagenicity results obtained with these hydrazides have been compared with mutagenicity results obtained with the corresponding nitrosamines and no quantitative correlations have been noted.

2,3,7,8-Tetrachlorodibenzofuran (TCDF) and the alkyl nitrites are currently being tested. Butter yellow and its two analogues are all mutagenic for TA1538, but have differing activities. In order to obtain the optimum mutagenic activity, increased levels of S-9 were needed. The Salmonella results obtained with butter yellow and its analogues will be compared with results obtained in other microbial and whole animal systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cyclic hydrazides are used as organic intermediates in organic synthesis reactions. This demonstration of their mutagenicity has implications for the health of workers handling these chemicals. It does not appear as if these hydrazides are the active metabolites of nitrosamines but they are potentially hazardous in their own right. TCDF and alkyl nitrites are substances to which the population is exposed. Examination of their mutagenicity will provide information that can be used in risk estimation procedures, should they become necessary.

Butter yellow and its analogues will provide structure/activity information that may be useful in predicting the toxicity of other, related substances.

PUBLICATIONS

Zeiger, E. and J. Guthrie (1981). Cyclic hydrazides are mutagenic for Salmonella typhimurium. Mutation Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 60105-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Genetic Control of Mutation

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

PI: J. M. Mason Geneticist LMG NIEHS

COOPERATING UNITS (if any)
B. Slatko, Department of Biology, Williams College, Williamstown, MA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.4	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

It has become evident in the last several years that mutation rate is under genetic control. In bacteria and yeast the frequency of induced mutations can be either increased or decreased by blocking one or another pathway of DNA repair. This project is designed to determine the relationship between DNA repair and mutagenesis in Drosophila melanogaster. Two approaches are being taken: (1) A mutant which increases the mutation frequency (a mutator) has been identified and is being characterized; and (2) The interaction of DNA repair defective mutants and hybrid dysgenesis (naturally occurring mutators) is being observed in double mutant combinations.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized mutants and chromosomal aberrations in Drosophila melanogaster are employed.

MAJOR FINDINGS AND PROPOSED COURSE: One mutator being examined is unable to repair X-ray induced breaks in the normal way. In the presence of the mutator broken chromosomes are recovered which appear to be deficient for a telomere [on both cytological and genetic grounds]. That is, unlike X-ray induced aberrations in wild type, the broken chromosomes do not appear to be capped by any previously existing telomere. The mutator is recessive, and maps to chromosome III. It is active throughout oocyte development, but does not appear to be active during spermiogenesis. The mutator does not increase the frequency of meiotic nondisjunction. It does, however, increase the frequency of X-chromosome loss. These losses are the results of whole arm deletions in which the centromere is recovered but the rest of the X has been lost.

Certain naturally occurring mutators ("hybrid dysgenic strains") have very specific phenotypes in terms of where in the genome they allow mutation, chromosome breakage or recombination to occur. It has been hypothesized that the genetic changes induced in the presence of hybrid dysgenesis are the result of DNA sequences inserting into or excising from genomic DNA. If this is the case the cell's DNA repair capacity may play a role in movement of these sequences. We are making combinations of DNA repair deficient mutants and hybrid dysgenic factors to look for quantitative changes in mutation, recombination and segregation. The most striking observation so far is that the recovery of the dysgenic factor from a hybrid male decreases from about $\frac{D1}{D5}$ 0.4 in the controls to almost zero in the presence of mei-41 or . This is true for three independent dysgenic factors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will lead to an understanding of the cellular mechanisms used to regulate the rates of mutation and chromosome breakage. It should in the long run allow one to sequence a telomere.

PUBLICATIONS

Mason, J.M.: Spontaneous mutation frequencies in mutagen-sensitive mutants of Drosophila melanogaster. Mutation Res. 72: 323-326, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60106-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cytogenetic Analysis of Mutagen-Sensitive Mutants

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

PI:	J.M. Mason	Geneticist	LMG	NIEHS
	N.N. Scobie	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

J.B. Boyd, Department of Genetics, University of California, Davis, CA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 pleio-tropic effects on related functions such as recombination. At the present time a fine structure map of the mei-41 region is being constructed in order to ascertain the allelism relationship of mus 104 and mei-41 as well as to confirm the large size of mei 41 found during mutational analysis.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized X-linked mutants and chromosomal aberrations in Drosophila melanogaster are employed. Because the mutagen-sensitive (mus) mutants are X-linked the presence of these mutants is monitored by mating mus males to attached-X females, treating the progeny with MMS (or other mutagen), and checking the sex ratio of the survivors.

MAJOR FINDINGS AND PROPOSED COURSE: Mutants in two putative mus loci are very similar in phenotype in that they are sensitive to the same mutagens and they are defective in post-replication repair. However, post-replication repair in mutants at one "locus" (mus 104) is sensitive to caffeine while post-replication repair in mutants at the other (mei-41) is not. It is not clear from the initial mapping nor from complementation studies whether these mutants are allelic. A fine structure map is being constructed to clarify the allelic relationship of these mutants. The results so far lead to the following conclusions. (a). Two mus 104 alleles map within the mei-41 locus and thus are allelic. (b). If mus 104 and mei-41 are allelic they cannot define two different genes or two different pathways of post-replication repair as proposed by Boyd et al. (c). Since mei-41 and mus 104 have different effects on meiosis but the same effect on sensitivity to mutagens it is possible to uncouple the effects of mutants of this locus in different tissues. The reason for this uncoupling may become evident after other alleles are added to the map of this region. (d). The mei 41 locus is very large in recombinational terms (0.25 centimorgans). It is the largest locus known in Drosophila and 50 X the size of a simple gene such as ry. This is consistent with the observation that mei 41 is about 25 X the size of a typical mus X-linked gene in a mutational study. Making the usual assumptions as to the genome size and the amount of recombination in Drosophila we calculate that the mei 41 locus contains about 145 kb and that if a protein product is made it should be about 5×10^6 daltons.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the action of genes controlling mutagen sensitivity is necessary for understanding DNA repair, mutagenesis, recombination and chromosome stability.

PUBLICATIONS

Mason, J.M., Green, M.M., Shaw, K.E.S., and Boyd, J.B.: Genetic analysis of X-linked mutagen-sensitive mutants of Drosophila melanogaster. Mutation Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60107-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Cloning and Sequence Analysis of Various Regions of the T4 Genome

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

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
Other:	M. A. Conkling	Geneticist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The bacteriophage T4 is a powerful tool in the analysis of basic mechanisms of mutagenesis. The genetics of the rII region have been extensively used and inferences of particular mutagenic pathways can be made. With molecular clonging and DNA sequencing techniques we can now confirm certain inferred pathways. Cloned sequences will also aid in the biochemical analysis of certain T4 functions involved in fidelity and repair. The genome of bacteriophage T4 will be cloned by recombinant DNA techniques. The primary cloning vector will be M13. The cloned sequences will be pooled and probed for various regions of interest either by DNA:DNA hydridization or by their ability to complement or recombine with T4 phage defective for the desired activity. These regions will then be sequenced or used in biochemical analyses.

PROJECT DESCRIPTION

METHODS EMPLOYED: Purified T4 DNA will be digested with restriction endonuclease TaqI. Vector M13 mp7 replicative form DNA is restricted by restriction endonuclease AccI and the generated ends are filled with dTTP. Both DNAs are annealed, ligated by DNA ligase and transformed by E. coli. Clones of interest will be selected by hybridization, complementation or marker rescue. Identified cloned DNA will be sequenced by Sanger's dideoxynucleotides termination methods.

MAJOR FINDINGS AND PROPOSED COURSE: We have been trying to sequence regions of the T4 rII genes without first cloning them. This approach uses an end-labeled restriction fragment of part of the rII gene. This fragment is annealed to the separated T4 DNA strands and act as a primer for DNA sequence method. However, T4 DNA is too long and causes high backgrounding. Meanwhile, it is discovered that restriction endonuclease TaqI can digest T4 DNA, unlike other restriction endonucleases. We are now developing a technique to clone TaqI-digested T4 DNA into a M13 vector.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Much of what we know of basic mechanisms of mutagenesis has been inferred from the genetic system of phage T4. The cloning and sequencing of certain mutants will allow us to verify proposed pathways of mutagenesis by environmental mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60108-03 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of DNA Replication in Prokaryotes

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

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
Other:	K. C. Kim	Visiting Fellow	LRDT & LMG	NIEHS
	J. Arendes	Visiting Fellow	LMG	NIEHS
	P. Carl	Guest Worker	LMG	NIEHS

COOPERATING UNITS (if any)
Dept. of Biophysics & Theoretical Biology, Univ. of Chicago, Chicago, IL
Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A) An in vitro replication system for bacteriophage N4 has been developed to study the mechanism of its DNA replication. The system mimics in vivo DNA replication. The DNA replication proceeds continuously from both ends of the DNA molecule as in vivo. By using this system, we have purified three DNA replication proteins to homogeneity and identified their functions.

B) The Guest Worker (P. Carl) has isolated a mutant which is deficient in RNaseH activity. It has been speculated that RNaseH removes RNA primer from "Okazaki Fragment". We have studied the RNaseH mutant genetically and biochemically. Also, we have been cloning the RNaseH gene to generate a much tighter mutant of RNaseH by in vitro mutagenesis.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: In vitro DNA synthesis system. Wild type and various DNA replication-negative N4 phages and the crude extracts from those phage-infected cells. Conventional column chromatographies and velocity sedimentation for purification of DNA replication proteins. Standard cloning technique. Restriction endonucleases. DNA-DNA and colony hybridization. RNaseH assay using RNA-DNA hybrid. Electron microscope, agarose and polyacrylamide electrophoresis, and electrofocusing gel.

MAJOR FINDINGS AND PROPOSED COURSE: A) N4 DNA replication. Coliphage N4 contains a linear double stranded DNA genome of 72 kb pairs and a DNA dependent RNA polymerase in its virions. N4 DNA replication does not require the activity of E. coli genes dnaA, dnaB, dnaC, dnaE, dnaG and polA. Mutants in at least eight N4 cistrons affect N4 DNA replication. One cistron codes for the virion RNA polymerase required for the synthesis of N4 early RNAs. The products of three other cistrons are required for transcription of N4 middle RNAs which code for at least four functions involved in DNA synthesis (DO). Moreover, a study of the behavior of temperature sensitive mutants in the virion RNA polymerase suggest that it also plays a direct role in N4 DNA replication. We have developed an in vitro system to study the mode of initiation of N4 DNA replication. N4 infected cells are gently lysed and the soluble proteins are concentrated by ammonium sulphate precipitation. DNA synthesis in this extract is totally dependent on the addition of exogenous native N4 DNA. N4 heat denatured and other DNAs are poor templates. No activity is detected in extracts derived from N4 DO mutant infection. However, mixing of extracts from two DO mutants, in different cistrons, restores activity to nearly wild type levels. We are using this complementation assay to purify the N4 coded components required for in vitro DNA synthesis. In the course of these experiments, the cistrons corresponding to the N4 coded DNA polymerase and DNA-binding protein have been identified and purified to homogeniety. In vitro N4 DNA replication starts from both ends of the DNA molecule and proceeds continuously as does in vivo. B) E. coli RNaseH. 1) The E. coli rnh gene will be cloned in order to confirm the map location we have assigned to this gene. Such a clone will probably be an overproducer of this important enzyme. 2) Using the cloned DNA and in vitro mutagenesis techniques we will construct potential RNase H mutants which will be propagated on plasmids. By inducing recombination between host and plasmid rnh sequences we plan to produce strains carrying solely mutant rnh sequences. Alternatively RNase mutants will be selected by classical in vivo techniques of localized mutagenesis. 3) The rnh mutants will be characterized with respect to their growth and macromolecular synthesis. In particular we shall examine DNA synthesis in the mutants to see if we can find evidence for an increased number of Okazaki pieces bearing RNA primers or changes in the length of primers. 4) The new rnh alleles will be combined with mutants such as polAex1 already known to affect the processing of Okazaki pieces to see if more severe defects in the processing are found in the double mutant.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, particularly the importance of replication protein complex, and will provide a precise understanding of mutagenic mechanisms.

PUBLICATIONS

Sugino, A., Higgins, N. P., and Cozzarelli, N. R.: Covalent attachment of the DNA gyrase A protein to DNA. Nucleic Acids Res. 8, 3865-3874 (1980).

ABSTRACTS

Risk, J. K., Sugino, A., and Rothman-Denes, L. B.: In vitro coliphage N4 DNA replication. ICN-UCLA Symposia, Molecular and Cellular Biology - Structure and DNA-Protein Interactions of Replication Origins. J. Supramol. Structure, Supl. 5, p. 340 (1981).

Sugino, A., Risk, J. K. and Rothman-Denes, L. B.: In vitro coliphage N4 DNA replication: Purification of N4 DNA replication proteins. Cold Spring Harbor Phage meeting (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60109-03 LMG

PERIOD COVERED

October 1, 1980 to September, 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of DNA replication in eukaryotes

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

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
Other:	H. Kojo	Guest Worker	LMG	NIEHS
	J. Arendes	Visiting Fellow	LMG	NIEHS
	K. C. Kim	Visiting Fellow	LMG	NIEHS
	B. D. Greenberg	Biologist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A) The Drosophila mutant which possesses an altered DNA polymerase α has been further characterized. Similar types of DNA polymerase mutants have been isolated from the yeast Saccharomyces cerevisiae and it has been proven that DNA polymerase I is a true DNA replicase in yeast.

B) An in vitro DNA replication system of yeast 2 μm plasmid has been developed from a crude extract of S. cerevisiae. The in vitro origin and direction of plasmid 2 μm DNA replication have been determined and are the same as in vivo.

C) In order to understand the importance of DNA tertiary structure for DNA replication, DNA topoisomerases, which might play an important role in DNA replication, have been purified from human and insect cell lines and studied extensively.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard DNA cloning technique. Chemical and enzymatic DNA nucleotide sequencing methods. DNA-sythesizing crude extract from wild type and various cdc mutants of yeast Saccharomyces cerevisiae.

Agarose and polyacrylamide gel electrophoresis, sucrose or glycerol density gradient sedimentation, and electron microscopy to analyze the products of in vitro DNA replication systems. Ethyl methane sulfonate mutagenesis using D. melanogaster cell line and yeast S. cerevisiae to isolate mutants which possess an altered DNA polymerase α and DNA polymerases of yeast, for mutant selection. Conventional column chromatographies and velocity sedimentation centrifugation for purification of DNA polymerases and other DNA replication proteins.

MAJOR FINDINGS AND PROPOSED COURSE: A) DNA polymerase α is mainly responsible for nuclear DNA replication. Isolation of DNA polymerase α mutants would be very useful for studying several aspects of DNA polymerase biochemistry, including the regulation of biosynthesis of the enzyme and its role in various cellular reactions such as DNA repair and recombination as well as DNA replication itself.

Recently, it has been shown that "aphidicolin" inhibits not only mitosis, but also in vivo DNA replication (particularly DNA polymerase α activity) in the sea urchin. Moreover, it has been shown that this drug also inhibits DNA replication in vivo and in vitro where DNA polymerase α might be involved in eukaryotes, including SV40, adenovirus, Drosophila nuclear DNA replication and yeast nuclear and 2 μm DNA replication.

This project focuses on the isolation of DNA polymerase genes from Drosophila. We have isolated aphidicolin-resistant DNA polymerase α mutant from a Drosophila melanogaster cell line and are continuing to try to isolate ts mutants from these mutants. Meanwhile, we have been trying to isolate DNA polymerase α gene using DNA (gene) transfer methods. B) Isolation of various DNA polymerase mutants in yeast: Although many ts mutants are already available in yeast and some have been identified as DNA replication mutants, none is a DNA polymerase mutant. Virtually all DNA polymerase activity in crude extracts from yeast is inhibited by aphidicolin, as is in vivo DNA replication.

The drug-resistant mutants have been isolated, and from these we will be able to isolate a subset of DNA polymerase ts mutants. Then using these resistant mutants, we will isolate the DNA polymerase gene and clone it in both E. coli and yeast by the following technique.

Assuming that drug resistance is dominant, nuclear DNA from the drug-resistant cells will be digested with various restriction endonucleases, and then linked to the yeast 2 μm-DNA vector or to the E. coli pBR322 vector; recombinants will be selected using conventional methods. In the case of E. coli, $polA^{ts}$, $polC^{ts}$ or $polA^{ts}$ and $polC^{ts}$ will be used as the host.

To select yeast recombinants containing the mutant DNA polymerase gene, total recombinants are grown on plates containing aphidicolin. To select E. coli recombinants containing the yeast DNA polymerase gene, total recombinants are

grown at restrictive temperatures. Finally, using radioactive antibodies against yeast DNA polymerase we can identify clones containing the yeast DNA polymerase gene. C) To understand the mechanism of DNA replication in eukaryotes, we have developed an in vitro DNA replication system of yeast 2-μm plasmid. This system mimics in vivo and starts DNA synthesis at the same site as in vivo and DNA synthesis proceeds bidirectionally. Some of the cell division cycle mutant crude extracts did not support significant in vitro 2-μm DNA synthesis under the restrictive condition. Therefore, this system provides better assay for purification of various DNA replication proteins and studying more precise mechanics of DNA replication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, and will provide a precise understanding of mutagenic mechanism.

PUBLICATIONS

Sugino, A., and Nakayama, K.: DNA polymerase α mutants from a Drosophila melanogaster cell line. Proc. Natl. Acad. Sci., USA. 77, 7049-7053 (1980).

Kojo, H., and Sugino, A.: Mechanism of action of aphidicolin in yeast Saccharomyces cerevisia. Nucleic Acids Res. in press (1981).

Kojo, H., Greenberg, B. D., and Sugino, A.: Yeast 2-μm plasmid DNA replication in vitro: Origin and direction. Proc. Natl. Acad. Sci., USA. 78 in press (1981).

Sugino, A., Kojo, H., Greenberg, B. D., Brown, P. O., and Kim, K. C.: In vitro replication of yeast 2-μm plasmid DNA. ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XXI (eds. D. S. Ray and C. F. Fox, Academic Press) in press (1981).

ABSTRACTS

Sugino, A., Kojo, H., Kim, K. C. and Greenberg, B. D.: The origin and direction of 2-μm yeast DNA replication in vitro, 10th Annual ICN-UCLA Symposia on Molecular and Cellular Biology, J. Supramol. Structure and Cellular Biochem. Suppl. 5 p. 345 (1981).

Nakayama, K., Sugino, A., and Cheng, Y. C.: Demonstration and Purification of DNA topoisomerases from HeLa cells. Fed. Proc. (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60110-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of the mutagenic action of hydroxylamines in bacteriophage T4.

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

PI:	P. R. Tempest	Visiting Fellow	LMG	NIEHS
Other:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS
	B. W. Glickman	Expert	LMG	NIEHS

COOPERATING UNITS (if any)

None

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0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Hydroxylamine and its N- and O-methylated analogues are potent mutagens which react with DNA to produce a variety of lesions. Cytosine is thought to be the primary mutagenic target but of the two (or three) cytosine adducts formed it is not clear which is the one responsible for the mutagenic event. The ratio of these products can be dramatically altered by changing pH, temperature, reagent concentration and more notably by the presence of a hydroxymethyl group at the C5 position of cytosine (C). The purpose of this study is (1) to determine whether alterations in the reaction products of hydroxylamines as predicted from available chemical data affect the rates of mutation and inactivation of bacteriophage T4; (2) to determine the frequency with which a hydroxylamine-modified residue in DNA mispairs in vivo; (3) to quantify the relative amounts of the products of reaction of hydroxylamine with T4 DNA containing either 5-hydroxymethylcytosine (5HMC) or C.

PROJECT DESCRIPTION

METHODS EMPLOYED: Bacteriophage T4 normally contains HMC residues in its DNA. However, with the appropriate genes rendered defective it is possible to obtain phage with biologically functional DNA containing unsubstituted C residues. Studying the hydroxylamine-induced reversion of rII mutants of T4 which contains either HMC or C residues offers the unique opportunity to examine, presumably without alteration of the replication mechanism, how the different reaction products of these two bases with hydroxylamines affect mutagenesis and inactivation.

The in vivo mispairing potential of aberrant residues in DNA during replication may be readily examined in T4 by examining the distribution of mutant and revertant phenotypes amongst the progeny of a singly-infected bacterium.

The approach used to detect hydroxylated cytosine residues in DNA will be to treat phage whose DNA has been prelabeled with ^{3}H-cytidylate residues with hydroxylamine, isolate and enzymatically hydrolyse the DNA, and separate the modified cytosine residues by standard chromatographic techniques.

MAJOR FINDINGS AND PROPOSED COURSE: We have determined from in vivo measurements that the lesion(s) primarily responsible for mutation in T4 DNA produce G·C → A·T with very high efficiencies per round of DNA replication. Furthermore, we have detected no difference between the efficiency of "mispairing" produced by the products of hydroxylamine or O-methyl-hydroxylamine in T4 DNA. This finding was not necessarily predicted on the basis of hydrogen-bonding models for mispairing during DNA replication, and suggests that factors in addition to hydrogen bonding potential need to be considered when predicting mutagenic properties of modified DNA bases.

Experiments in which $N^{4}OH$ cytidine was expected to be the major mutagenic product (when DNA contains 5-hydroxymethyl C) of hydroxylamine, and experiments in which an additional di-adducts of cytosine are expected (in DNA containing unmodified C), did not show a major difference in the frequency with which mutational heterozygotes produced mutations during DNA replication.

Other experiments, examined the specificity of mutation produced by $N^{4}OH$ cytidine when supplied as an analogue to T4-infected E. coli. Substantial mutagenesis was found for both G·C → A·T and A·T → G·C transition pathways. The ability to produce both transitions is expected for conventional base analogues, and has been found for 5-bromouracil or 2-aminopurine. Recent reports in the literature indicated that in T4 (Sledziewska and Janion, Mutation Res. 70, 11-16, 1980) and in E. coli (Janion and Glickman, Mutation Res. 72, 43-47, 1980) this analogue produced primarily (only) A·T → G·C mutations. The observation of the single transition pathway in T4 appears to have been due to inadequate detection of the G·C → A·T transitions due to selection artifacts. The reasons for observing only a single pathway of mutation in E. coli may be different, however. We intend to further investigate the E. coli response to the analogue, and to attempt to substantiate that the analogue is used as a base analogue in T4 by investigating the role of the T4 DNA in producing mutations in the presence of T4 previously grown in the presence of the analogue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Hydroxylamine is widely used by geneticists as a means of producing mutants. Despite a large number of chemical studies on the reactivity of hydroxylamine with bases and ribonucleosides the precise lesion responsible for its mutagenicity has often only been inferred from these data and *in vitro* misincorporation assays using RNA polymerase. We hope that our *in vivo* experiments will define more clearly the primary mutagenic lesion(s) and to provide an explanation as to why and how frequently mispairing events of modified C residues occur during DNA replication.

PUBLICATIONS

Tempest, P. and L. S. Ripley, The properties of hydroxylamine mutagenesis in bacteriophage T4, in preparation.

Ripley, L. S., The mutation specificity of N^4OH cytidine in bacteriophage T4, in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60111-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the role of gene 43 DNA polymerase in frameshift mutagenesis

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

PI:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS
Other:	N. B. Shoemaker	Microbiologist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

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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 (200 words or less - underline keywords)

DNA polymerase of T4 plays a major role in spontaneous frameshift mutation. Using the T4 rII genetic system for measuring mutation, we have identified a number of polymerase mutant alleles which strongly increase frameshift mutation frequencies. The spectrum of frameshift production is unique to the polymerase mutation. To date, genetic positions of frameshift mutations have been determined and correlated to the wild type DNA sequence. The existence of the potential for the formation of DNA secondary structure in one frameshift region suggests a novel DNA structural precursor to frameshift mutation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulation of bacteriophage T4 utilizing mutants in the rII genes to measure mutation rates and frequencies, and mutations in the DNA polymerase gene 43 to alter fidelity of DNA metabolism. Definition of frameshift mutations in the T4 rII region requires the DNA sequencing of rII mutants. Cloning of T4 DNA is technically complicated because of the presence of modified cytosine in the DNA which inhibits the cutting of T4 DNA by most restriction enzymes. We are developing methods which will allow us to sequence a number of DNA mutants without transfering each mutant to a gentic background (containing four additional unlinked mutations) to provide DNA in which C is not modified. (See # Z01 ES 60107-03 LMG)

MAJOR FINDINGS AND PROPOSED COURSE: DNA polymerase plays a major role in frameshift mutation. Both the total frequency of frameshift mutation and the specific nature of frameshift mutations promoted is unique to specific DNA polymerase mutant alleles. We have examined the spectrum of rIIB mutations in bacteriophage T4 produced by the L98, L56, L88, and L141 alleles of gene 43 the T4 DNA polymerase enzyme. Frameshift mutations to date have been characterized with respect to their genetic location, their genetic sign, and in some cases with respect to their spontaneous and proflavin-induced reversion frequencies. We next plan to characterize the mutations with respect to their DNA sequences. The wild type DNA sequence of the region is already known, and the genetic location of the mutations is suggestive of their location in particularly interesting DNA sequences. The sequencing will confirm the exact location and will define the exact deletion or addition character of the mutations.

One interesting DNA sequence is ATTGGCTGATTGGC. Two frameshifts in this region might be the addition or deletion of 8 BP from this sequence resulting from misalignment of DNA. Furthermore, this sequence lies in a larger region of DNA (about 35 BP) which has the potential of forming an imperfect hairpin structure of unusually high stability (approximately-14Kcal). We plan to investigate the possibility that this structure may be a structural precursor to frameshift mutation in this piece of DNA.

A mutant T4 polymerase allele, A58, has been identified in our initial studies as producing a strong increase in frameshift mutation in the rIIB gene. This allele in previous tests has not been shown to strongly influence fidelity of base pair substitution mutations in T4. We intend to determine the spectrum of frameshift mutation enhanced by A58, and further to determine its effect upon base pair substitution mutation. It is likely that this allele represents a unique example among the T4 polymerase alleles investigated to date of the specific perturbation of frameshift fidelity without a concommitant change in basepair substitition fidelity.

The mutant T4 polymerase allele, L141, increased the frequency of frameshift mutation at some sites, while decreasing the frequency of frameshift mutation at other sites. This same polymerase has the property of decreasing certain base pair substitution mutations (spontaneous A·T → G·C transitions and base analogue induced transitions) while increasing other mutations (spontaneous transversion

base pair substitutions). We plan to determine the characteristics of this enzyme which distinguish it's "mutator" from it's "anti-mutator" effects.

Frameshift mutation has been known for many years to be strongly influenced by DNA sequence. We have now shown that T4 DNA polymerases strongly influence the frequency of frameshift mutation in a site specific manner. Frameshift mutation frequencies can also be enhanced by acridines such as proflavin. We plan to investigate whether the frameshift mutations promoted by proflavin are primarily mediated by DNA sequence properties, DNA polymerase properties, both, or other as yet undefined properties.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Frameshift mechanisms are not understood. General models have been proposed but no specific enzymology impinging on these mutations is well characterized. Frameshifts represent a particularly large fraction of "detected" spontaneous mutations in procaryotic systems. The reason for this is at least two-fold. First, frameshifts are efficiently detected compared to missense mutations. Frameshift mutations generate multiple missense codons after the frameshift and frequently generate a prematurely terminated peptide. Thus frameshift mutations can have an extremely deleterious effect in protein coding DNA sequences. Second, spontaneous frameshifts show a strong sequence preference. Genes carrying such hot spots show high spontaneous frameshift frequencies. The lysozyme gene of T4 and the rII genes of T4 have frameshift hotspots consisting of runs of A·T basepairs. The lac I gene of E. coli has a frameshift hot spot consisting of a tetranucleotide sequence repeated three times in the wild type sequence. Both addition and deletion frequencies are high in those hotspot sequences in which they can be distinguished.

Many frameshift mutations in protein coding regions of eucaryotic organisms including man may well be recessive because of their extremely deleterious effect. Thus, such mutations might contribute heavily to the genetic load of the population. The importance of frameshift mutation in non-protein coding sequences is unknown.

PUBLICATIONS

Ripley, L. S. and N. B. Shoemaker, Polymerase infidelity and frameshift mutation, in Molecular and Cellular Mechanisms of Mutagenesis, Ed. by J. F. Lemontt, and W. M. Generoso. Plenum Press, NY (1981).

Ripley, L. S., The specificity of infidelity of DNA polymerase in the XIV, Rochester International Conference on Environmental Toxicity, Induced Mutagenesis: Molecular mechanisms and their implications for environmental protection in press (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60112-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular mechanism of mismatch correction in E. coli

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

PI:	B. W. Glickman	Expert	LMG	NIEHS
Other:	M. Babcock-Harms	Guest Worker	LMG	NIEHS
	G. Moessen	Biologist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Our approach has relied on standard mutagenesis analysis as well as the analysis of mutational specificity in the lacI gene of E. coli. Transfection experiments using heteroduplexed λ DNA were carried out to measure mismatch correction in wild-type and repair-deficient strains of E. coli.

MAJOR FINDINGS AND PROPOSED COURSE: In recent years a major error-avoidance pathway responsible for the removal of misincorporated bases during DNA replication has been identified. The mechanism involves the recognition and excision of mismatched bases and differentiates between the "correct" parental and error-containing daughter DNA strands on the basis of DNA methylation levels, the key to discrimination being that parental DNA strands are fully methylated and daughter DNA strands are, following DNA replication, non-methylated. Mutants of this error-avoidance pathway which have been characterized are the dam mutant, defective in DNA methylation with the resulting loss of strand discrimination, and the mutator mutants mutH, mutL and mutS which control an early step in mismatch base excision, probably incision itself. Genetic characterization of these mutants shows that they, along with uvrE, uvrD and recL, belong to the same repair pathway. The level of mutagenesis in a multiple mutant is the same as in a mutH mutant and is about 10,000-fold higher than in the wild-type strain. This suggests that the total error-rate in E. coli, about one error in 10^{10} nucleotides 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 influence of mismatch repair on the directionality and specificity of mutagenesis by base analogs. The amber hotspots seen during 2-aminopurine mutagenesis are also evident in mutH strains suggesting that their prevalence above other sites are not related to mismatch repair. Studies with N^4OH-cytosine which shows an AT → GC preference are also underway in the mismatch repair strains.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The identification and analysis of this error-avoidance pathway will contribute greatly to our understanding of the mechanism by which the cell maintains such a high level of fidelity during DNA replication.

PUBLICATIONS

Brouwer, J., G. Mohn and B. W. Glickman. Dam^+ is required for mutagenesis by methylating agents. Mutation Research (in preparation).

Glickman, B. W. 3. DNA repair and its relationship to the origin of human cancer. In: Genetic Origins of Tumor Cells; F. J. Cleton and J. W. I M. Simons (editors) Nijhoff, the Jague, p. 25-51 (1979).

Glickman, B. W. Spontaneous mutagenesis in Escherichia coli strains lacking 6-methyl-adenine residues in their DNA: an altered mutational spectrum in dam⁻ mutants. Mutation Research 61, 153-162 (1979).

Glickman, B. W. and M. Radman. Escherichia coli mutator mutants deficient in methylation-instructed DNA mismatch correction. P. N. A. S. Vol. 77, 1063-1067 (1980).

Guijt, N. and B. W. Glickman. UV protection in mutator strains of E. coli. J. Bacteriol. (in preparation).

Todd, P. A. and B. W. Glickman. UV protection and mutagenesis in uvrD, uvrE and RecL strains of Escherichia coli K12 carrying the pKM101 plasmid. Mutation Research, 62, 451-457 (1979).

Glickman, B. W. Methylation-instructed mismatch correction as a post-replication error avoidance mechanism in Escherichia coli in Molecular Mechanisms of Mutagenesis, ed. A. Hollander (1981).

Janion, C. and B. W. Glickman. N^4-hydroxycytidine: a mutagen specific for AT to GC transitions. Mutation Research 72, 43-47 (1980).

Mohn, G., N. Guijt and B. W. Glickman. The influence of dam⁻ and the mutator plasmid pKM101 on mutational response: implication for mutagen screening. Mutation Research 74, 255-265 (1980).

Knaap, A. G. A. C., B. W. Glickman and J. W. I. M. Simons. Effects of ethionine on the replicational fidelity in V79 chinese hamster cells. Mutation Research 82, 355-363 (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60113-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular mechanisms of mutagenesis in E. coli

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

PI:	B. W. Glickman	Expert	LMG	NIEHS
Other:	R. Dunn	Biologist	LMG	NIEHS
	C. Felton	Biologist	LMG	NIEHS

COOPERATING UNITS (if any)
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Laboratory of Molecular Genetics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project is designed to achieve a better understanding of how the cellular repair capacity, the nature and extent of the DNA damage and cellular metabolism interact to determine the biologically important endpoints of survival and mutagenesis. The work reported in this project involves the genetical and biochemical characterization of the DNA repair processes involved in error avoidance and error fixation. In particular, we have investigated the affect of dose on mutational specificity in order to learn more about what mutational events occur as different cellular repair capacities become saturated. This data not only will provide a basis for an improved understanding of the mechanism by which mutation occurs but will also lay the groundwork for a more accurate understanding of low-dose effects and their associated risks.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The determination of mutagenic specificity requires the isolation and characterization of hundreds of independently occurring mutations. The principles of the technique can be stated as follows: 1) the isolation of large numbers of independently occurring lacI$^-$ mutants; 2) the determination of nonsense mutations by suppression analysis; 3) separation of nonsense mutations into groups on the basis of localization by mapping data and 4) the correlation of each mutation with a specific mutational site by an analysis of the suppression pattern in strains carrying well characterized nonsense suppressors. This process requires the use of special techniques for the rapid detection and analysis of mutants on a large scale. In order to facilitate 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.

A second approach involves the sequencing of revertants of amber mutants of bacteriophage M13. The phage can be treated in vitro and mutagenesis can be carried out in "SOS" induced and non-induced host cells.

MAJOR FINDINGS AND PROPOSED COURSE: X-rays: Following a dose of 30kR the specific locus rate was 4.5×10^{-10} mutations per rad per gene copy per cell and the nucleotide substitution rate was 2.2×10^{-12} per rad. At the doubling dose (4kR) the mutational spectrum contained a greater fraction of 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.

UV-mutagenesis: An analysis of the mutation spectrum for UV light showed that most if not all mutagenesis occurred at sites where pyrimidine dimers could be formed. Several mutational hotspots were correlated with secondary DNA structure. An analysis of the dose response for various sites in the spectra demonstrate that different hotspots respond with different kinetics: spectra then appear quite different at different UV-fluences.

Akylating agents: Preliminary experiments were carried out to determine the effect of cellular repair capacity and dose on the mutagenic and toxic effects of alkylating agents. The role of the recA, lexA, umuC, uvrA, uvrB, uvrC, recF, recL, uvrD and uvrE genes upon the repair of EMS and MMS induced damage was examined. In this way we were able to separate direct muational effects and indirect effects: Moreover, we found that a functional uvrA, uvrB, uvrC repair capacity was required

for the excision of ethylation but not methylation base damage. The alk mutation results in hypermutability by EMS and also alters the lacI MMS spectrum.

Initial mutation specificity experiments with EMS and MMS show: 1) The mutational spectre are dose dependent. For example, in the case of the wild-type strain a low dose of MMS increased the mutation frequency by a factor of 50 and resulted in about 30% amber and ochre mutants among the lacI⁻ mutants. However, while doubling this dose increased the induced mutation frequency by a factor of two, of this mutant population only 4.5% were ambers and ochres. This demonstrates that the mutants produced at the higher dose were qualitatively different from those produced at the lower dose. 2) The mutational spectrum of EMS is primarily GC to AT base substitutions. In the uvrB and dam⁻ strains, however, all four transversion events were uncovered at significantly high frequencies. The molecular basis for the altered mutational spectra for EMS in these repair-deficient strains is being further investigated. 3) The mutational spectrum for MMS in the wild-type cell differs from that caused by EMS and MNNG in that both transitions and transversions are detected. This strengthens the idea that MMS works by a mechanism distinct from that of other alkylating agents. The production of the full spectra for MMS at a broad range of doses whould help to clarify this question, particularly if the mutational spectra for these agents in the repair-deficient strains is also ascertained. 4) The mechanism of mutagenesis by MMS is thought to involve both direct and indirect effects. However, we have found the dam⁻ strain to be non-mutable by MMS. This suggests a role for DNA repair mechanisms in the fixation of MMS damage which was hitherto unsuspected.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will lead to an improved knowledge of how DNA repair systems affect the mutational response and assist in clarifying the molecular basis for mutagenesis. Moreover, data are being obtained which will enable the assessment of risk estimates to be made using knowledge of molecular specificity rather than solely on the basis of empirical extrapolation.

PUBLICATIONS

Brouwer, J., N. Guijt and B. W. Glickman. The repair of alkylation damage in E. coli K12: dam and alk involve different repair pathways. J. Bacteriol. in preparation.

Glickman, B. W., K. Rietveld and C. S. Aaron. λ-ray induced mutational spectrum in the lacI gene of Escherichia coli; Comparison of induced and spontaneous spectra at the molecular level. Mutation Research 69, 1-12 (1980).

Janion, C. and B. W. Glickman. N^4-hydroxycytidine: a mutagen specific for AT to GC transitions. Mutation Research 72, 43-47 (1980).

Mohn, G., N. Guijt and B. W. Glickman. The influence of dam⁻ and the mutator plasmid pKM101 on mutational response: implication for mutagen screening. Mutation Research 74, 255-265 (1980).

Schaaper, R. and B. W. Glickman. UV-induced mutagenesis: a prerequisite for a pyrimidine dimer at the target site? Science (submitted)

Todd, P. A., J. Brouwer and B. W. Glickman. EMS and MMS mutagenesis in DNA repair deficient mutants of E. coli K12. Mutation Research 82, 239-250 (1981).

Todd, P. A. and B. W. Glickman. UV protection and mutagenesis in uvrD, uvrE and RecL strains of Escherichia coli K12 carrying the pKM101 plasmid. Mutation Research, 62, 451-457 (1979).

Todd, P. A., C. Monti-Bragadin and B. W. Glickman. MMS mutagenesis in strains of Escherichia coli carrying the R46 mutagenic enhancing plasmid: phenotypic analysis of Arg$^+$ revertants. Mutation Research 62, 227-237 (1979).

Glickman, B. W. Methylation-instructed mismatch correction as a post-replication error avoidance mechanism in Escherichia coli in Molecular Mechanisms of Mutagenesis, ed. A. Hollander (1981).

Glickman, B. W. Mutational specificity of UV light in E. coli: influence of excision repair and the mutator plasmid pKM101. Symposium volume of the 14th Rochester International Conference on Environmental Toxicity (1981).

Glickman, B. W. Altered mutational specificity with dose of γ-rays. Third International Conference on Environmental Mutagens, Tokyo, Japan (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60114-02 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Activation of Polycyclic Hydrocarbons and other compounds to Mutagenic products by Prostaglandin Synthetase

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

PI:	I. Robertson	Visiting Fellow	LMG NIEHS
	E. Zeiger	Supervisory Microbiologist	LMG NIEHS
	T. Eling	Head, Prostaglandin Group	LPFT NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Function and Toxicology, NIEHS

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Prostaglandin synthetase is capable of metabolizing a number of substances previously thought to be metabolized only by the mixed-function oxidase system. A method has been developed for testing the ability of prostaglandin synthease to metabolise xenobiotics to mutagenic products.

Benzo(a)pyrene, benzanthracene, chrysene and a number of their metabolites have been tested for mutagenicity after activation by prostaglandin synthetase.

A number of aromatic amines and related compounds including the bladder carcinogen benzidine, and nitrosamines are in the process of being tested.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test has been modified to circumvent the toxicity of the detergent used to solubilize the prostaglandin synthetase and of the arachidonic acid used as a cofactor in the reaction. A crude microsomal preparation containing prostaglandin synthetase is prepared from ram seminal vesicles. This preparation is sterilized by filtration and included in a pre-incubation modification of the standard Salmonella plate test.

MAJOR FINDINGS AND PROPOSED COURSE: 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 metabolised 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 cytochrome P-450 system as represented by Aroclor-1254 induced rat liver S-9. The cytochrome P-450 system, unlike the prostaglandin synthetase system, was also able to activate the parent compounds and several other dihydrodial derivatives. Thus, the prostaglandin synthetase system appears more selective than the cytochrome P-450 system in the conversion of polycyclic hydrocarbons and their metabolites to mutagenic products.

Having demonstrated the ability of prostaglandin synthetase to activate representative polycyclic hydrocarbons, we are now proceeding to examine the ability of this system to activate aromatic amines and related compounds and nitrosamines.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most current research on the activation of xenobiotics is with cytochrome P-450 systems. Prostaglandin synthetase is ubiquitous throughout mammalian tissues and frequently co-exists with cytochrome P-450. There is increasing evidence that prostaglandin synthetase may serve as an alternative or complementary activation system. Our work will help to further elucidate this role of prostaglandin synthetase in the metabolic activation of xenobiotics.

PUBLICATIONS

Guthrie, J., I.G.C. Robertson, E. Zeiger, J. Boyd, and T. E. Eling. Activation of several polycyclic aromatic hydrocarbons to mutagenic products by prostaglandin synthetase. Cancer Res. (to be submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60115-02 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Study of Rates Of Reaction of Styrene Oxide Isomers Using Mutagenicity Testing

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG	NIEHS
	D. Pagano	Research Microbiologist	LMG	NIEHS
	J. Bend	Supervisory Pharmacologist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology, NIEHS

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.35	0.15	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Salmonella typhimurium tester strain TA100 is being used in a study of the reaction rates of styrene oxide optical isomers. Previous studies have shown that enantiomers (optical isomers) of some compounds differ in their production of mutation, carcinogenicity, or their interactions with activating or detoxifying enzymes. This study has shown that styrene oxide, a compound with industrial significance, has optical isomers which differ in their mutagenicity levels while maintaining similar rates of mutation production. Studies will be continued using reconstituted enzyme systems in order to determine if this difference exists where conjugation with glutathione is followed using mutation as the endpoint. Testing of styrene oxide conjugates; styrene glycol, styrene-glutathione, styrene-cysteinyl-glycine, styrene-cysteine and styrene-mercapturic acid showed no mutagenic or toxic responses in the absence of S9 activation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Salmonella/microsome test is being used with a modification of the pre-incubation procedure to allow timed samples to be examined.

MAJOR FINDINGS AND PROPOSED COURSE: Dose responses of styrene oxide (+), (-) isomers and racemic (±) showed similar mutagenicities over a dose range 0 to 30 μmoles/plate. A reproducible trend in the results suggests that the (-) compound is a more potent mutagen followed by the racemic and (+) isomer compounds. Precipitation and toxicity levels were similar in all 3 compounds. In a timed pre-incubation study (0 to 120 minutes pre-incubation), all 3 compounds showed similar rates of mutagenicity as evidenced by the shape of curves generated over the full pre-incubation interval. The major difference among the 3 compounds was the level of mutagenicity at each time point. Again the (-) isomer appeared to be the more potent mutagen, followed by the racemic and (+) isomer, in that order. The racemic mutagenic level was approximately equidistant between the levels of the optical isomers.

The addition of cytosol from uninduced rat liver S9, decreased the mutagenicity of all 3 compounds. This was not due to non-specific protein binding since no such decrease in mutagenicity was seen when styrene oxide was mixed with bovine serum albumin at equivalent protein concentrations. The decrease was probably due to the presence of endogenous glutathione and glutathione-S-transferases in the cytosol preparations. Experiments are proposed which will use a reconstituted system, i.e. purified glutathione-S-transferase and glutathione in order to determine if the isomers differ in their rates of conjugation with glutathione. Also, since the Salmonella tester strains have been shown to have endogenous levels of glutathione, attempts will be made to develop a tester strain which is deficient in glutathione production.

Conjugates of styrene oxide, e.g. styrene glycol, styrene-cysteinyl-glycine, styrene-cysteine, styrene-gluthathione and styrene-mercapturic acid were tested for mutagenicity in the absence of rat liver S9. All compounds were not mutagenic. There was insufficient material to test these compounds in the presence of S9.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Styrene is an important industrial chemical which is also carcinogenic. The mammalian metabolites of styrene are mutagenic for Salmonella. This study is attempting to differentiate between the biological activities of the (+) and (-) isomers of styrene oxide as a means of studying their mechanisms of action in vivo. It has also shown that the conjugates of styrene which appear in the urine do not possess any direct ability to produce mutations or toxicity in Salmonella.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60116-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of a Disc Method for the Rapid Control and Identification of Ames Tester Strains

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG NIEHS
	D. Pagano	Research Microbiologist	LMG NIEHS
	I. Robertson	Visiting Fellow	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A simple scheme has been developed for confirming the phenotype of the standard set of Salmonella typhimurium tester strains. This scheme employs a series of filter paper discs impregnated with diagnostic mutagens or bacterial toxins. Up to 6 diagnostic discs can be placed on a petri dish to test a single Salmonella strain. The Salmonellae are distinguished by their responses to ampicillin, crystal violet, nitrofurantoin, 9-aminoacridine, 4-nitro-o-phenylenediamine and sodium azide. The discs have maintained their reactivity in storage for 13 months.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard Salmonella spot test procedure of Ames is used.

MAJOR FINDINGS AND PROPOSED COURSE: It is possible, using various combinations of the discs with each strain, to identify the strain and to confirm the strain's genotype (i.e., rfa, pKM 101, base pair/substitution, frameshift). Strains TA-98 and TA-100, which contain the plasmid pKM101 are resistant to the toxic effects of ampicillin; all strains should contain a deep rough (rfa) mutation and are, therefore, all sensitive to the killing effects of crystal violet. Only TA-100 should be mutagenized by nitrofurantoin; sodium azide will mutagenize both TA-100 and TA-1535. 9-Aminoacridine is specific for TA-1537, however, 4-nitro-o-phenylenediamine will mutagenize both TA-98 and TA-100. This method is rapid and easy to use and provides identification and confirmation of the tester strains at the time of their use in mutagenicity testing. Impregnated discs have remained stable under the proper storage conditions longer than 13 months. Monitoring the stored discs will continue to determine their storage life.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Ames/Salmonella test enjoys widespread use in laboratories of all types and sizes. Controls (positive mutagens) should be run at all times. In addition the strain's genotypes should be checked at all times. The method developed here will eliminate excess handling of mutagens (carcinogens) for control plate tests, would provide a fast and inexpensive positive control system and allow for the positive identification of each strain used at the time of its use.

PUBLICATIONS

Zeiger, E., D. Pagano and I.G.C. Robertson (1981). A Rapid and Simple Scheme for confirmation of Salmonella Tester Strain Phenotype. Environ. Mutagen. 3: in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60117-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Study of Tris (2,3 dibromo propyl phosphate) and its Metabolities for Mutagenicity

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG NIEHS
	D. Pagano	Research Microbiologist	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.05	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The Salmonella strains TA-100 and TA-1535 have been used to test for the mutagenicity of Tris-BP, a known carcinogen, and some of its metabolites. The chemical structures of a series of metabolites were determined and the metabolites were synthesized at NIEHS. A gradient of mutagenic responses was seen when the metabolites were tested. These responses ranged from very weakly positive to as mutagenic as the parent compound. All positive responses required Aroclor-induced rat liver S-9. Testing trimethylphosphate (a direct-acting mutagen) and tripropylphosphate (a non-mutagen) supported the fact that the leaving group, 2-3 dibrompropyl phosphate, is responsible for TRIS-BP mutagenicity with the metabolite, 2-bromopropene, contributing some additional mutagenicity. Both compounds are direct-acting mutagens.

PUBLICATIONS

Zeiger, E., D. Pagano and A.A. Nomeir. Structure/Activity Studies on the mutagenicity of Tris (2,3-Dibromopropyl) Phosphate (Tris-BP) metabolites. Environ. Mutag. (submitted)

PROJECT DESCRIPTION

METHODS: Standard Salmonella/microsome plate test procedures were used.

MAJOR FINDINGS AND PROPOSED COURSE: The Tris-BP metabolites produced a gradient of mutagenic responses when tested at doses up to 200 nmoles/plate. Two metabolites, $C_7H_{12}Br_3O_4$ and $C_7H_{13}Br_4O_4P$, gave mutagenic responses equivalent to those obtained from the parent compound; one metabolite, $C_7H_{11}Br_2O_4P$, gave approximately one-half the Tris-BP response, while a $C_5H_{11}Br_2O_4P$, compound was about one-fourth the level. All reactions required metabolic activation with Aroclor-1254 induced rat liver S-9 implying that these substances are not the ultimate mutagens formed from Tris-BP.

Tripropylphosphate was also tested to confirm the ultimate need for bromine groups. This compound was not mutagenic with or without S-9 implying a need for bromine groups. Trimethylphosphate was also tested to determine if the PO_4 ester core of the molecules might be contributing to the mutagenicity. Trimethylphosphate was found to be directly mutagenic in contrast to the Tris-BP metabolites which required metabolic activation. Also while Tris-BP and its metabolites were mutagenic in TA100 and TA1535, trimethylphosphate was only mutagenic in TA100. The results of this work have been submitted for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The levels of mutagenicity for the various metabolites suggest that they may be produced by a detoxification pathway. Since Tris-BP is a known carcinogen/mutagen, it would be of interest to know the degree of structural modification needed for its complete detoxification. Identification and testing of Tris-BP metabolites will provide an understanding of the mechanism of action of Tris-BP, provide evidence that can be used in inter-species comparisons and allow the design of chemical structures which are not capable of being metabolized to mutagenic products. The appearance of a pattern to the structural changes and decreasing mutagenicity suggests a detoxification pathway of Tris-BP in rats. The important feature of this pathway is that the dibromopropyl side groups are removed sequentially and the 3-bromo moiety can be removed with the resulting formation of a 2-bromopropene side group. Both of these side groups are known mutagens. Extrapolation of this data to humans is, therefore, possible and is supported by the fact that 2,3-dibromopropyl groups can be identified in the urine of children exposed to Tris-BP. An understanding of the mechanism of action of Tris-BP provides evidence that inter-species comparisons may be made and allow design of chemical structures which are not capable of being metabolized to mutagenic (and possible carcinogenic) products.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60119-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Influence of Age on the Metabolic Activation of Carcinogens to Products Mutagenic to Salmonella

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

PI:	I. Robertson	Visiting Fellow	LMG NIEHS
	L. Birnbaum	Research Microbiologist	TRTP NIEHS
	E. Zeiger	Supervisory Microbiologist	LMG NIEHS

COOPERATING UNITS (if any)

Environmental Biology Branch, NIEHS
Center for Human Aging, Duke University Medical Center (Dr. R. Cooper)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

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 (200 words or less - underline keywords)

Salmonella typhimurium tester strains are of use in examining the metabolic activation of chemical carcinogens using mutation as the endpoint. This project has determined the influence of age on the metabolic activation of carcinogens by preparations from liver, lung and kidney of female rats of different ages and determine the influence of pituitary tumors which occur with high frequency in senescent female rats of this strain.

PROJECT DESCRIPTION

METHODS EMPLOYED: Tissue preparations from female Long-Evans rats of different ages were incorporated in a standard Salmonella plate test.

MAJOR FINDINGS: Age-related changes in drug metabolism of the liver, lung and kidney of adult female Long-Evans rats were determined by measuring changes in mutagen formation. Activation of aflatoxin B_1, 2-aminofluorene, and 2-acetylaminofluorene to mutagenic derivatives was assayed using the Ames Salmonella test system. The promutagens were incubated with tissue extracts from rats ranging in age from 2 1/2 to 25 months. With all three compounds, hepatic, renal, and pulmonary activation was lower in senescent than in young adult animals. The largest decrease, however, occurred prior to middle-age, that is, before 9-13 months. In liver and kidney, little change was detectable between the middle-aged and the old (20-25 months) animals. However, pulmonary metabolism in the oldest animals was slightly higher than in the extracts from the middle-aged rats. The observed decline in mutagen activation may thus be a function of maturation rather than senescence.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolites of many procarcinogens are thought to be the ultimate carcinogens in mammals. The findings of this project will give information concerning the hypothesis that some fraction of the markedly increased incidence of neoplasia observed in senescent mammals is a result of age-related alterations in the metabolism of chemical carcinogens.

PUBLICATIONS

Robertson, I.G.C. and L.S. Birnbaum. Changes in Mutagenic Activation with Senescence in Rat Tissues. Chem.-Biol. Interactions (submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60120-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolic Activation of Known Carcinogens by Rabbit Lung to Products Mutagenic to Salmonella

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

PI:	I. Robertson	Visiting Fellow	LMG NIEHS
	E. Zeiger	Supervisory Microbiologist	LMG NIEHS
	R. M. Philpot	Research Chemist	LP NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.60	0.55	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Salmonella typhimurium tester strains have been used to examine the metabolic activation of chemical carcinogens by rabbit liver and lung cytochromes using mutation as the endpoint. This project will determine the ability of different forms of cytochrome P450 present in lung tissue to activate known carcinogens to mutagenic metabolites. It will also allow a comparison to be made on the relative metabolic activating abilities of lung and liver.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Both S_{16} supernatant and microsomal fractions from rabbit lung and liver are incorporated in standard Salmonella plate and/ or pre-incubation tests. Metabolic activation is further characterized using monooxygenase systems, reconstituted from purified components, in the Salmonella test. Such characterization includes the effects of antibodies to, and inhibitors of, specific forms of cytochrome P450.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary work was aimed at identifying which carcinogens of interest are mutagenic to Salmonella using rabbit pulmonary activation. Two nitrosamines, DMN and nitrosomorpholine, are inactive and two polycyclic aromatic hydrocarbons, benzo(a)pyrene and 3-methylcholanthrene, are only weakly active with rabbit pulmonary S-16. However, the aromatic amines, 2-aminoanthracene (2AA), 2-aminofluorene (2AF) and 2-acetylaminofluorene (2AAF) and the fungal toxin aflatoxin B_1 (AFB_1) are active.

These agents are more readily metabolized to mutagenic products by 16,000 x g supernatant fractions from pulmonary homogenates than by similar hepatic fractions despite the low pulmonary cytochrome P-450 content of this tissue, and more revertants per nmol total cytochrome P-450 were consistently obtained with pulmonary than with hepatic microsomal preparations.

In reconstituted monooxygenase systems containing the major pulmonary cytochrome P-450 isozymes ($P\text{-}450_I$ or $P\text{-}450_{II}$), $P\text{-}450_{II}$ was highly effective in the activation of 2-aminoanthracene and 2-aminofluorene and also active with 2-acetylaminofluorene, whereas these substrates were not activated by $P\text{-}450_I$. This difference was confirmed by the results of antibody inhibition studies carried out with pulmonary microsomal preparations. The higher activity of pulmonary relative to hepatic preparations can be accounted for by the relatively high proportion of $P\text{-}450_{II}$ in the lung (approximately 50% total cytochrome P-450 content) as compared to the liver (less than 5%). However, antibody to $P\text{-}450_{II}$ did inhibit the hepatic microsomal activity by 50-70% indicating that $P\text{-}450_{II}$ is important in the activation of these agents in both tissues even though it is a minor component in the liver.

Aflatoxin B_1 was only activated by $P\text{-}450_I$ in reconstituted monooxygenase systems although the antibody inhibition studies indicated activation by both $P\text{-}450_I$ and $P\text{-}450_{II}$ in microsomal preparations.

Other carcinogens are in the process of being tested. Once mutagenicity is established the metabolic activation will be further characterized to identify the relative roles of $P\text{-}450_I$ and $P\text{-}450_{II}$.

A significant difference in the metabolism of 2-aminofluorene and 2-acetylaminofluorene has been observed. The basis of this difference is being examined using deacetylase inhibitors and hepatic microsomes prepared from animals induced by phenobarbital and B-napththoflavone.

The metabolism of the aromatic amines to mutagenic products is, to date, the only activity identified for P-450$_{II}$, but not P-450$_{I}$, in the rabbit lung. This activity is therefore being used as a marker at different stages in the purification of P-450$_{II}$.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
The susceptibility of the lungs to many chemical carcinogens that are not carcinogens in the liver raises intriguing questions as to basis of difference. Both organs contain P450-dependent monooxygenase systems which have been implicated in the metabolic activation of carcinogens.

PUBLICATIONS

Robertson, I.G.C., R.M. Philpot, E. Zeiger and C.R. Wolf. Specificity of Rabbit Pulmonary Cytochrome P-450 Isozymes in the Activation of Several Aromatic Amines and Aflatoxin B_1. Molecular Pharmacology (submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60121-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of Computerized Sucrose Gradient Analysis System

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

PI:	M. Resnick	Research Geneticist	LMG NIEHS
	M. Rowley	Computer Systems Analyst	BB NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.15	.10

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The ongoing research program on DNA repair and meiosis in yeast involves extensive analysis of DNA changes during meiosis and after treatment with mutagens. The primary means of assessing changes is through the use of sucrose gradient analysis of large molecular weight chromosomal DNA. Because of the extensive data and calculations required, we are developing an interactive computer program to store and analyze data directly from the scintillation counter with several options for mode of analysis and means for comparison by utilizing the NIEHS PDP11 computer, the PROPHET system, and RS1.

PROJECT DESCRIPTION

METHODS EMPLOYED: A non-interactive program for analyzing sucrose gradient results obtained from Dr. Jay Donniger, NCI and Dr. Richard Setlow, Brookhaven National Laboratory for modifications and updating.

MAJOR FINDINGS AND PROPOSED COURSE: The program has been modified to enable interactive use by the investigator. Data from the scintillation counter will be directly transmitted via a TI 700 ASR terminal to the NIEHS PDP11. The program has been developed to enable rapid evaluation of molecular weights and gradient profiles and will have plotting capabilities to enable comparisons between various experiments; the output of tables and graphs will be in a form ready for publication.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This program will greatly aid in the analysis of data generated during the course of studies on mechanisms of DNA repair during mitotic and meiotic growth and will greatly assist in developing data for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60122-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of DNA Repair in Yeast

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

PI:	M. Resnick	Research Geneticist	LMG NIEHS
	S. Stasiewicz	Biological Laboratory Technician	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

DNA repair in mitotically growing cells of the yeast Saccharomyces cerevisiae is under the control of more than 20 genetic loci. The products of some genes have been shown to be involved in normal meiosis. We have developed sucrose gradient techniques for the examination of repair events in mitotically growing cells and meiotically developing cells after low doses of UV and ionizing radiation to wild type and repair-defective strains. Using these techniques we are determining the role of mitotically identified repair functions on damage occurring during meiosis. We have demonstrated that wild type cells efficiently excise UV-induced pyrimidine dimers both as mitotically growing or meiotic cells. Excision is blocked in a rad1 throughout meiosis suggesting that there are no additional excision repair systems during meiosis. As found for mitotic growth, DNA synthesis proceeds past UV-induced pyrimidine dimers during meiosis indicating that there is a general ability to bypass lesions in the DNA. The bypass mechanism does not appear to involve recombination. In fact, the presence of damage appears to inhibit the meiotic round of genetic recombination whereas it stimulates recombination in mitotically growing cells.

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 radioactivly-labeled cells are examined for the appearance of breaks using sucrose gradient techniques and for the repair of UV-induced pyrimidine dimers. The presence of UV-induced pyrimidine dimers is indicated by sensitivity of the DNA to the endonuclease activity in M. luteus extract which is specific for pyrimidine dimers. In studying the role of repair mechanisms during meiosis, wild-type and repair-deficient strains are tested at various times for recombination and plating efficiency as well as the appearance of DNA strand breaks.

MAJOR FINDINGS AND PROPOSED COURSE: Meiosis is a fundamental developmental stage which occurs in nearly all eukaryotes. Although there is considerable information on the genetic and morphological changes that take place, relatively little is known about DNA metabolic events, DNA repair, or mechanisms of mutation. Since DNA metabolism and recombination in meiotic cells are different from those processes in mitotic cells, the repair capabilities might be expected to differ considerably from those in mitotically growing cells. For example, there may be repair mechanisms that are unique to meiosis or there may be unique levels of repair capabilities. This research represents an integrated attempt to examine DNA repair mechanisms in a well-characterized mitotic/meiotic system at both the molecular and genetic level.

We have developed a lysis and sucrose gradient technique which enables the identification of full-size chromosomal DNA in yeast. A recently improved method, which involves a gentle lysis of cells and is not affected by the post-irradiation or meiotic fragility of cells, allows for the detection of less than 1-2 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. Using complete and also "leaky" excision defective mutants, we have demonstrated that pyrimidine dimers do not act as complete blocks to DNA synthesis. Both meiotic and mitotic cells have the capacity to efficiently replicate past them. It appears that the replication past dimers does not involve recombination of large regions of DNA, contrary to results with bacteria. Eukaryotes generally lack the bacterial type recombinational repair mechanism. The molecular evidence with meiotic cells correlates well with genetic results in that the presence of damage during meiosis appears to suppress meiotic recombination. As part of this study, we have also demonstrated that wild type cells can efficiently remove dimers throughout meiosis and that the only excisional repair mechanism is that associated with the rad1 pathway identified originally in mitotic cells. Using these techniques we also plan to investigate the repair of damage due to other types of agents, during mitotic growth and meiosis particularly low levels of ionizing radiation and various mutagenic

agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: DNA repair mechanisms are of fundamental importance in the process of mutagenesis and ultimately in carcinogenesis. Using yeast as a model lower eukaryote, we have been able to dissect and analyze at least two pathways of DNA repair at the molecular level in growing and meiotic cells. Since these pathways are involved in mutagenesis, this work will further our understanding of the basic mechanism of mutation. In addition this work enables a genetic and molecular examination of the importance of DNA damage in mitotic and meiotic systems and the relevance of DNA repair in these two stages of development.

PUBLICATIONS

Resnick, M.A., Boyce, J., Cox, B. Postreplication repair in yeast. J. Bacteriol. (in Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60123-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

DNA Repair Processes During Meiosis

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

PI:	M. Resnick	Research Geneticist	LMG NIEHS
	S. Stasiewicz	Biological Laboratory Technician	LMG NIEHS

COOPERATING UNITS (if any)

Dr. Robert Roth, Dept. of Biology, Illinois Institute of Technology, Chicago, IL and Dr. John Game, Dept. of Genetics, U. of California, Berkeley, CA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Using the yeast Saccharomyces cerevisiae we are examining mitotically identified DNA repair systems during normal meiosis. 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 and rad52 mutants, recombination is abolished and for rad52 the spore products are inviable. The rad52 mutant accumulates single-strand DNA breaks/gaps during meiotic DNA synthesis in both the parental and newly synthesized strands. The timing corresponds to the beginning of lethality: lethality and breaks are prevented by inhibitors of DNA synthesis. The frequency of breaks (about 200 per cell) approximates the number of genetic recombinational events. We conclude that the RAD52 gene product is involved with recombination and the breaks may be intermediates in this process. The breaks probably occur prior to homologous chromosomes pairing. It thus appears that the rad52 gene product is essential in normal meiosis and in protection from ionizing radiation damage; the mechanism of action involves recombination.

PHS-6040
(Rev. 2-81)

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 radioactivly-labeled cells are examined for the appearance of breaks using sucrose gradient techniques. In studying the role of repair mechanisms during normal meiosis, wild-type and repair-deficient strains are tested at various times for recombination and plating efficiency as well as the appearance of DNA strand breaks.

MAJOR FINDINGS AND PROPOSED COURSE: In the yeast Saccharomyces cerevisiae DNA repair processes are required in mitotically growing cells to protect against external damaging agents and most of the repair mechanisms are involved in mutagenesis. We are investigating the role of various repair systems during the meiotic stage of development in terms of their importance to normal meiosis. Mutations in the rad6 genes; which is required for UV-induced mutagenesis, do not prevent meiotic DNA synthesis; however, meiotic recombination does not occur nor are meiotic products produced. 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 radl 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. (We are currently studying this using ligase defective strains). Unlike the wild-type, the rad52 mutants accumulate single-strand breaks 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. 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 that the 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 single-strand breaks or gaps. It is interesting that lethality in the rad52 mutant follows the appearance of the DNA interruptions.

These results are the first report of any mitotically identified DNA repair mutant being examined at the molecular level during meiosis. Furthermore, this is the first report of any mutation affecting meiosis which leads to specific changes during meiosis other than gross alterations in DNA synthesis. We are expanding this work to examine the nature of the recombinational events and possible sits of recombination. Recent work has indicated that the rad52 meiotic changes are associated with early steps in recombination not involving paired chromosomes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Little is known about the role of DNA repair mechanisms during the meiotic development of eukaryotes. With the techniques we have developed, yeast affords the opportunity to examine at both the genetic and the molecular level the importance of various repair systems during normal meiosis and following challenges by various mutagens during meiosis. The yeast system may also serve as a relevant model for understanding events in the germ lines of whole animals wherein, for technical reasons and lack of genetic systems, many of these studies cannot be conducted.

PUBLICATIONS

Game, J.C., Zamb, T.J., Braun, R.J., Resnick, M. and Roth, R.M.: The role of radiation (rad) genes in meiotic recombination in yeast. Genetics, 94 (1980): 51-68.

Resnick, M.A., Kasimos, J.N., Game, J.C., Braun, R., and Roth, R.M.: Changes in DNA during meiosis in a repair-deficient mutant (rad52) of yeast. Science (in Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60124-02 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

An Aneuploidy Test System in Yeast

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

PI:	M. Resnick	Research Geneticist	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (x) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A contract(s) to be awarded in FY 1981 will provide for the development of an aneuploidy test system in the yeast Saccharomyces cerevisiae. It is expected that the contractor(s) will be able to develop a microbial system 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 utilized by the Environmental Mutagenesis Test Development Program to detect genetically active agents.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The monitoring of this contract will follow standard contract procedures and will utilize the expertise of the PI to assure a high level of scientific development.

MAJOR FINDINGS AND PROPOSED COURSE: Based on proposals received, it will be possible to develop and validate a system of yeast strains which will enable the detection of several genetic endpoints in addition to aneuploidy. The system(s) should be adaptable to continue testing situations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aneuploidy contributes significantly to the genetically based disease burden in human populations with approximately 0.4% of live births exhibiting abnormal chromosome numbers. A large fraction of spontaneous abortion in humans and certain serious genetic diseases (e.g., Down's syndrome) are caused by aneuploidy. Aneuploidy has also been implicated in some steps in tumor promotion. A few chemicals are known to induce aneuploidy specifically in test systems; however there is in fact, no reliable, well-developed rapid screen to detect such agents on a large scale. A yeast test system will enable the future rapid screening of chemicals and agents 1) that induce gross chromosomal changes which would not be identified as mutagenic in microbial test systems, and/or 2) cause changes in chromosomal number in addition to being mutagenic.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60127-01 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Use of the Intra-sanguinous Host Mediated Assay to Study Organ-Specific Mutagenicity of Chemicals

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

PI:	E. Zeiger	Supervisory Microbiologist	LMG	NIEHS
	D. Pagano	Research Microbiologist	LMG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The intrasanguinous host-mediated assay is being used to study the metabolism of chemicals to mutagenic products in different organs of mice. Bacterial or yeast cells are injected into the tail vein and can be recovered from the liver, kidneys, lungs and testes. Initial studies with chemicals are underway.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The testing procedure used is the intra-sanguinous host-mediated assay. An indicator strain is injected into the tail vein of mice and the chemical being tested is given orally or by IP or IM injection. After *in vivo* incubation, the mice are sacrificed and the tester strain is recovered from various organs by excision, homogenization, dilution and plating of the homogenerates onto complete plates for survival and minimal media plates for enumeration of mutant colonies.

MAJOR FINDINGS AND PROPOSED COURSE: Initial studies were carried out in order to determine the distribution and survival of the bacterial tester strains after tail vein injection of approximately 10^9 cells. Our studies have shown that one hour after injection approximately $2x10^8$ cells/ ml can be recovered from the liver, $1\text{-}3x10^7$ cells/ml can be recovered from the lungs and kidney and approximately $3x10^5$ cells/ml from the testes. Follow-up studies allowing for 2 and 4 hour periods of time after injection of 10^9 cells showed only a slight increase in cell recovery in the liver and testes and no change or a slight decrease in cell recovery from the lungs and kidney after 2 hours but increases in cell recoveries from all four tissues after 4 hours; i.e. $1x10^9$ cells/ml in liver, $5\text{-}6x10^7$ cells/ ml in lung and kidneys and $2x10^6$ cells/ml in testes. These studies were carried out using *Salmonella typhimurium* strains with intact cell walls. Similar studies using the standard *Salmonella* strains with cell wall deficiencies showed that after one hour incubation following tail vein injection, cells could be recovered only from the liver (approx.0.1%).

Studies with dimethylnitrosamine (DMN), a known liver mutagen and carcinogen, have been performed using this method. The results show that after a single, sub-lethal dose of DMN a high level of revertant bacteria could be recovered from the livers of dosed mice when compared to solvent controls.

Studies are planned for testing a series of structurally-related nitrosamines which have known target-organ carcinogenicity in order to study the ability of this short-term test to detect target-organ specificity. Also, work on bacterial cell recovery from other organs will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The host-mediated assay provides the opportunity for detecting the effects of *in vivo* metabolism after chemical exposure by a number of different routes, using mutation induction in injected microorganisms as an endpoint. Additionally, the potential for determining target organ specificity adds a dimension to short-term testing that is currently only available in chronic long-term bioassays.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 60128-01 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Collaborative Study to Test for "Genetic Drift" in Laboratory Stocks of Ames' Strains

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

PI: I. Robertson Visiting Fellow LMG NIEHS

COOPERATING UNITS (if any)

Genetic Toxicology Section, ICI Ltd., U.K.

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

As part of an international study our stocks of the Salmonella typhimurium tester strains were compared with referee strains in their response to a reference mutagen, 4-nitroguinoline-N-oxide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Our own cultures and a set of reference cultures were tested with 4-NQO under the same conditions using a set protocol. Strains were also checked for known characteristics.

MAJOR FINDINGS: The experiments and strain characterizations have been completed and the results submitted to Dr. Anderson (ICI Ltd. UK) for compilation and analysis.

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 properties.

It will also give an indication of the extent of intra- and inter-laboratory variation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60129-01 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Nucleotide sequence variability surrounding origin of replication of human mitochondrial DNA

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

PI:	B. D. Greenberg	Biologist	LMG	NIEHS
Other:	A. Sugino	Visiting Scientist	LMG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

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 (200 words or less - underline keywords)

Mitochondria, cellular organelles present in all known eukaryotic cells, contain a semi-autonomous genetic system -- a unique quality among metazoans. In mammalian cells, there is a marked conservation of genetic function and arrangement within the mitochondrial DNA (mtDNA) molecule, which is completely and symmetrically transcribed, and encodes either proteins or the protein-synthesizing apparatus of the mitochondria. The remaining portion of the mtDNA contains the origin of DNA replication and the only two known origins of transcription. Moreover, there is economy of nucleotide base sequence within a species, although intraspecific variability does exist as seen by detailed restriction endonuclease analyses. Other investigators have located the greatest degree of polymorphism to the region which contains the replicational and transcriptional control sequences. It has been our goal to characterize this variability at the level of nucleotide sequence. We have cloned this polymorphic region of human mtDNA isolated from several different individual placentas, performed restriction mapping and hybridization analyses to define the precise origin of replication within the cloned segments, and begun DNA sequencing of the proposed variable regions.

PROJECT DESCRIPTION

METHODS EMPLOYED: The desired segment of mtDNA was a previously defined 3.0 Kb KpnI fragment. This fragment was isolated and ligated into a KpnI site of the chimeric plasmid pAD23 which contains pBR322 plus the HindIII D fragment of Adenovirus-2 DNA. Positive clones were identified by standard colony hybridization techniques with minor modification.

Restriction maps of these inserts were prepared by standard methods including single, double, and triple digestions, partial digestion, end labeling analyses and Southern hybridization. The origin of replication was defined by labeling and isolating "7S DNA" from the native mtDNA preparations on sucrose gradients. This small single stranded DNA represents a putative replication intermediate of mtDNA whose 5'-terminus is complimentary to the H-strand replication origin of the native molecule. Hence hybridization to restriction degests of the insert identified this sequence within the clones.

Clone fragments were prepared for sequencing by the low melting point agarose method and nucleotide sequencing is being performed by the method of Maxam and Gilbert.

MAJOR FINDINGS AND PROPOSED COURSE: Comparative restriction mapping has defined two types of sequence variability within this region of the mtDNA molecule. Single base alterations appear throughout this segment as documented in several mtDNA systems, and an additional insertion or deletion of approximately 100 bases occurs downstream from the replication origin, 300 to 700 bases from this point. This has not been previously observed.

We are proceeding with the nucleotide sequence analysis to resolve all of these issues. Additional comparisons will be possible with the full mtDNA sequence recently presented by Anderson, et. al. (Nature 290 (1981), 457-465) and the partial sequences overlapping this region of the molecule presented by Crews, et. al. (Nature 277 (1979), 192-198) and Crews and Attardi (Cell 19, (1980), 775-784). Only preliminary sequence data is presently available and must be confirmed prior to drawing any conclusions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Several investigators have suggested that the rate of evolution of the mtDNA molecule exceeds that of the chromosomal DNA. It is not known whether this reflects intrinsic mitochondrial processes such as permitted or purposeful base substitutions, or a lack of response to externally generated base changes, ie. inefficient correction mechanisms responding to mutagenic events. As an approach to resolving this question we must first define the nature of the intraspecific sequence variability. Conclusions concerning randomness or specificity of sequence polymorphism must await these results, with direct bearing on mechanisms of mtDNA diversification.

Furthermore, since this polymorphic region contains the control sequences for the initiation of replication and transcription, these results may have a bearing on these processes, illuminating the requirements for the corresponding enzymatic recognition sequences.

Hence these studies will contribute to the understanding of the complexity of DNA replication and gene transcription, and provide evidence concerning the mechanisms generating the sequence variability in the mammaliam mtDNAs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60130-01 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less).

Isolation of E. coli mutants defective in repair of alkylated DNA

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

PI: M. Volkert Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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. The initial selection method has been to isolate mutants which are incapable of supporting growth of bacteriophage damaged by alkylating agents. Such mutants are now being screened for sensitivity to alkylating agents. By this method, we expect to accumulating strains containing mutations in the enzymes responsible for repair of alkylated DNA as well as mutations in genes which regulate these enzymes or permit their expression. Such mutants will permit the systematic evaluation of the relative roles these repair enzymes perform in the recovery from alkylation damage, evaluation of the mutagenic properties of the lesions upon which specific enzymes act and identification of the genes which code for these enzymes.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic and microbiological techniques will be used for mutagenesis, mutant selection and isolation, and bacteriophage propagation.

MAJOR FINDINGS AND PROPOSED COURSE: The basic dosimetric measurements have been made for Ethyl Methane Sulfonate (EMS) treatment of both single stranded DNA and double stranded DNA phage. Optimal conditions for their use as a selective agent have also been established. A number of strains resistant to EMS treated bacteriophage have been isolated. They are now being tested for sensitivity to EMS in order to determine if they are in fact unable to repair DNA damage inflected by this agent simply resistant to the phage. When sufficient numbers of mutants sensitive to EMS have been isolated the mutations responsible will be mapped and the strains grouped on the basis of potential gene identity. This will allow the identification of the genes involved in coding for the enzymes which repair alkylated DNA and the subsequent identification of their products, their *in vivo* effects on all 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 alkyation damage to DNA act, what their effect is to permit cells to tolerate alkylation damage to DNA. How these enzymes are regulated at the genetic level and how repair mitigates or promotes mutagenesis in response to specific types of DNA damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60131-01 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Inhibition of postirradiation DNA degradation in UV damaged cells of E. coli

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

PI:	M. Volkert	Senior Staff Fellow	LMG	NIEHS
Other:	M. Hartke	Biological Aid	LMG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Inhibition of DNA degradation (DNA protection) in UV damaged cells of E. coli is thought to result from the production of an inhibitor which either protects single stranded regions of DNA or blocks the action of exonucleases, primarily ExoV, directly. Induction of the inhibitor of ExoV depends upon two genes, recA and lexA. Current hypotheses state that high levels of recA protein are expressed after UV damage. The levels of recA protein are sufficiently high to allow it to bind and protect the many single stranded regions present after replication of damaged DNA. Our previous studies have shown that rnmA mutant strains are not defective in ExoV activity nor do they produce high levels of recA protein either constitutively or after DNA damaging treatment. Nonetheless, rnmA strains do not degrade their DNA after UV irradiation. We are examining the nature of DNA protection after irradiation in rnmA strains, identifying the genes responsible and the proteins they produce. Since the recA gene is known to possess regulatory properties the possibility exists that is role in postirradiation DNA protection in wild type strains is regulational rather than functional. These experiments will provide us with an opportunity to examine this question.

PROJECT DESCRIPTION

METHODS EMPLOYED: DNA synthesis and degradation will be monitored by radioactive label incorporation into DNA and loss of acid precipitable label after UV treatment. Cell survival assays, strain construction, mutant isolation will be performed using standard genetic and microbiological techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Contrary to current theories of how DNA is protected from exonuclease activity after UV irradiation we find no requirement for high levels of recA protein in rnmA strains of E. coli rnmA mutants either poses an alternative mechanism which prevents postirradiation DNA degradation or have suffered a mutation which has uncoupled the DNA protection mechanism from recA protein induction. Some questions we now need to answer are: (1) Do recA- derivatives of rnmA strains block postirradiation DNA degradation. (2) Are any other recA dependent processes functional in recA- rnmA mutant strains? (3) What is the mechanism of postirradiation DNA protection in rnmA strains, what genes/proteins are involved? (4) Are the mechanisms of postirradiation protection identical in rnmA mutants and wild type strains?

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The cells ability to cope with damage to DNA is central to the understanding of how DNA damaging agents act to kill the cell and how damage results in the production of mutations. The relative effectiveness of error free mechanisms of repair is determined at least in part by the amount of substrate with which to carry out the repair. An understanding how cells protect damaged DNA from degradation will provide a better understanding how damaged DNA is metabolized and how it is preserved in order for repair to occur.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60132-01 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of the E. coli recF gene in DNA repair and mutagenesis

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

PI:	M. Volkert	Senior Staff Fellow	LMG	NIEHS
Other:	M. Hartke	Biological Aid	LMG	NIEHS

COOPERATING UNITS (if any)
Alvin J. Clark and Linda J. Margossian, Department of Molecular Biology, University of California, Berkeley, CA 94720

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.9	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Mutations in the recF gene causes a reduction in the expression of the SOS response. Increasing the levels of recA protein in recF mutants, either by introducing a multicopy plasmid bearing the recF gene or a recA operator constitutive mutation, has no effect on the recF phenotype. However, introduction of a recA mutation (tif-1) which produces a recA protein which, upon incubation at 42°C, expresses the recA proteolytic activity required for expression of the SOS response suppresses the UV sensitivity due to recF. This suggests that recF may be required for the activation of the protease activity of the recA protein. We are now investigating this possibility.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic and microbiological techniques are employed for strain construction, genetic manipulation, and cell survival and mutation frequency determination. recA protein levels are assayed by polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: The recF phenotype is not due to its regulatory effects on recA protein levels but may be due largely to its effect on the activity of recA protein. We are currently examining what aspects of the recF phenotype are suppressed upon the introduction of a recA gene which produces an activated form of recA protein and under what conditions this suppression occurs.

The effects of recF on induced mutagenesis and repair of single stranded DNA phage implicate recF as either a specific regulator of SOS repair or a protein required for its activity. Therefore, an understanding of the function of the recF gene product will provide insight into the regulation and mechanisms of SOS repair.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The recF gene is one of several genes involved in the expression of mutagenesis in E. coli via the inducible error prone DNA repair pathway. Its role in this process is not understood. Our study is designed to elucidate to regulatory and/or enzymatic roles of the recF gene product in order to gain a better understanding of inducible DNA repair systems and induced mutagenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60133-01 LMG

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Bisulfite Mutagenesis in Bacteriophage T4

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

PI:	J. W. Drake	Chief, LMG	LMG	NIEHS
Other:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Bisulfite, a common food preservative, has been previously reported to be mutagenic in a number of cellular systems and also in phage T4. Because phage T4 employs 5-hydroxymethylcytosine instead of ordinary cytosine in its DNA, however, and because 5HMC is chemically far more resistant to the mutagenically significant action of bisulfite normally observed with cytosine itself, the action of bisulfite upon T4 should be critically re-examined. Preliminary experiments have demonstrated a virtually complete nonreproducibility of the earlier T4 report in several major respects. Future work will explore the mutagenicity of bisulfite on T4 containing cytosine in its DNA instead of 5HMC.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The induced reversion of well characterized T4 rII mutants is tested with bisulfite under a wide variety of conditions in order to determine whether bisulfite is at all mutagenic in ordinary T4 particles. Using a special strain carrying multiple genetic defects in 5HMC biosynthesis, T4 particles are also prepared containing cytosine instead of 5HMC in their DNA, and the bisulfite-induced revertibility of rII mutants is then examined in these particles. Where necessary, cellular hosts are used that are unable to repair bisulfite-induced premutational lesions.

MAJOR FINDINGS AND PROPOSED COURSE: The major result to date is the irreproducibility of a previous claim of bisulfite mutagenicity in T4. A test will next be made of the ability of bisulfite to induce mutations in T4 particles bearing DNA containing cytosine instead of 5HMC.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Bisulfite is a putative mutagen which, paradoxically, is used as a food preservative. Its mutagenic potency and specificity should therefore be determined as a part of the process of estimating its potential risk to humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60134-01 LMG

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Methylmethanesulfonate Mutagenesis in Bacteriophage T4

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

PI: J. W. Drake Chief, LMG LMG NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

MMS is an alkylating agent with a distribution of preferred sites of methylation of nucleic acid bases quite different from that of several other monofunctional methylators, and in cellular systems MMS has always required the operation of an "error-prone repair system" to be mutagenic. In phage T4, however, MMS displays a dual mechanism, acting partly through the viral WXY error-prone repair system and partly independently of that system. WXY-dependent mutagenesis produces frameshifts and a variety of base-pair substitutions, as do other mutagens acting through this system (such as UV and gamma radiations). WXY-independent MMS mutagenesis produces mutations only at G:C base pairs, and these probably consist of transitions. The second mechanism is consistent with the observed (but infrequent) guanine-O^6 methylation by MMS, and the differences between phage T4 and cellular systems is consistent with a progressive increase in DNA polymerase discrimination associated with progressive decreases in mutability with increasing genome size.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: MMS-induced reversion is measured in T4 rII mutants previously closely characterized as to available paths of reversion; and in a converse approach, MMS-induced rII mutants are characterized for revertibility by mechanism-specific diagnostic mutagens such as proflavine, 2-aminopurine, 5-bromouracil and hydroxylamine. MMS-induced forward and reverse mutation is also carried out in genetic backgrounds defective in WXY-mediated error-prone repair, and in other genetic backgrounds likely to give information about induced mutagen specificities.

MAJOR FINDINGS AND PROPOSED COURSE: The major finding to date has been that MMS operates through two distinct mechanisms, one requiring error-prone processing of premutational lesions and the other acting independently of this system. This line of work is nearing completion and will be published soon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The monofunctional methylating and ethylating agents have been powerful and highly instructive models for understanding chemically induced mutation and carcinogenesis. The mutagenic properties of MMS as displayed in phage T4 may provide insights into the mutagenic process in more complex mammalian systems.

1. CASE WESTERN RESERVE UNIVERSITY - Cleveland, Ohio
(NO1-ES-9-2136)

2. EG&G MASON INSTITUTE - Rockville, Maryland
(NO1-ES-9-2137)

3. SRI INTERNATIONAL - Menlo Park, California
(NO1-ES-9-0001)

TITLE: Microbial Mutagenesis Testing

CONTRACTOR'S PROJECT DIRECTOR: 1. William Speck, M.D.
2. Stephen Haworth, Ph.D.
3. Kristien Mortelmans, Ph.D.

PROJECT OFFICER: Errol Zeiger, Ph.D., Supervisory Microbiologist
Environmental Mutagenesis Test Development Program

DATE CONTRACT INITIATED: 1. December 22, 1978
2. December 29, 1978
3. February 1, 1979

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 1125 environmental and commercial chemicals for mutagenicity using Salmonella typhimurium tester strains in 3 laboratories. Based on results in Salmonella chemicals are selected for chemical analysis and further testing in Drosophila, for cytogenetic effects in cultured mammalian (Chinese Hamster ovary) cells, and for other genetic testing.

METHODS EMPLOYED: Salmonella typhimurium strains TA-98, TA-100, TA-1535, and TA-1537 are being used to test for mutagenicity using a modification of the Ames Salmonella microsome assay. All chemicals are incubated with tester strains in suspension prior to addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation is provided by liver S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats and Syrian Hamsters. All chemicals are tested blind at 5 doses, in triplicate, in each Salmonella strain. Also, all chemicals are retested at least one 2 week following the first test. Results are being entered on data forms and transferred to a computerized data-base system or directly into a minicomputer for transfer to the data-base system.

MAJOR FINDINGS AND PROPOSED COURSE: Results have been received from these laboratories on a total of 363 test samples to date. It is anticipated that an additional 170 samples will be completed this calendar year, and that 300 samples will be tested per year in future years.

In addition, numerous requests for information and data on specific chemicals tested have been received from government personnel and from the private sector. All information requested has been provided.

Manuscripts are currently being written to present results of the initial chemicals in reviewed scientific journals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
These contracts will allow the NTP to rapidly screen large numbers of chemicals for mutagenicity in a relatively short time and at relatively low cost. Mutagenicity in this system correlates strongly with carcinogenicity and heritable mutations in rodents. The chemicals found mutagenic, therefore, will be given a high priority for chronic toxicological and carcinogenesis testing by the National Toxicology Program.

1. COLUMBIA UNIVERSITY - New York, New York
(NO1-ES-9-0014)

2. LITTON BIONETICS, Inc. - Kensington, Maryland
(NO1-ES-9-0013)

TITLE: In Vitro Cytogenetic Testing

CONTRACTOR'S PROJECT DIRECTOR: 1. Arthur Bloom, M.D.
2. Sheila Galloway, Ph.D.

PROJECT OFFICER (NIEHS): Errol Zeiger, Ph.D., Supervisory Microbiologist
Michael A. Resnick, Ph.D., Research Geneticist
Kenneth A. Palmer, Ph.D., Research Geneticist,
Division of Toxicology, FDA

DATE CONTRACT INITIATED: 1. September 29, 1979
2. September 29, 1979

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to develop and validate a protocol for testing a total of 350 chemicals for their ability to induce chromosome aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells. In order to do this, the contractors are required to standardize and validate a protocol.

METHODS EMPLOYED: Chinese hamster ovary cells in culture are being used to test for the induction of chromosome aberrations and sister chromatid exchange in vitro, both with and without S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats. The protocol has been developed and validated by the test laboratories. Results obtained from testing the unknown substances will be entered on standardized data forms and transferred to a computerized data base management system.

MAJOR FINDINGS AND PROPOSED COURSE: Eleven control chemicals including known carcinogens have been tested with and without S-9 activation in a blind study to validate the protocol. This protocol reconciles problems encountered earlier that related to cell viability. Results from the two laboratories show excellent comparability. Minor modifications have been made in the protocol and the laboratories have begun to test unknown compounds.

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.

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. Seymore Abrahamson, Ph.D.
2. Stanley Zimmering, Ph.D.
3. Ronald Woodruff, Ph.D.

PROJECT OFFICER (NIEHS): James Mason, Ph.D., Geneticist

DATE CONTRACT INITIATED: 1. September 28, 1979
2. September 28, 1979
3. September 28, 1979

CURRENT ANNUAL LEVEL: 1. $160,280
2. $131,333
3. $157,741

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 60 environmental and commercial chemicals for mutagenicity using Drosophila melanogaster tester strains in three laboratories. Substances which are found to be mutagenic in Drosophila will be selected for testing in mammalian systems.

METHODS EMPLOYED: Standard sex-linked recessive lethal and reciprocal translocation tests in Drosophila melanogaster are being used to test for mutagenicity. Chemicals will be selected based on results obtained from previous mutagenicity tests using Salmonella. Chemicals will be administered by feeding and the sex-linked recessive lethal test will be performed. If the results are negative, the test will be repeated after injection. If the results are again negative, the chemical will be considered nonmutagenic in Drosophila. If the results are positive, the chemical will be tested in the reciprocal translocation test using the means of administration which gave the positive result. In the reciprocal translocation test sperm will be stored to enhance the ability to recover chromosome breaks induced by the chemicals. Results will be entered on data forms and transferred to a computerized data base system.

MAJOR FINDINGS AND PROPOSED COURSE: Results have been received from

these laboratories on a total of eight test samples to date. It is anticipated that an additional 50 samples will be tested this calendar year.

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.

DEPARTMENT OF ENERGY
LAWRENCE LIVERMORE LABORATORY
UNIVERSITY OF CALIFORNIA
LIVERMORE, CALIFORNIA 94550

TITLE: Mutagens from the cooking of foods.

PROJECT DIRECTOR: Frederick T. Hatch, MD

PROJECT OFFICER (NIEHS): Errol Zeiger, Ph.D., Supervisory Microbiologist

DATE AGREEMENT INITIATED: 1. September 22, 1978 (222-YO1-ES-80038)
2. April 1, 1981 (222-YO1-ES-10063)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this interagency agreement are to identify the mutagens produced in foods cooked under approximately normal household conditions and determine their mechanism(s) of formation, assess the spectrum of genetic toxicity caused by these mutagens using in vitro and in vivo short-term tests, devise strategies to limit or prevent mutagen formation and to estimate the normal dietary intake of these mutagens.

METHODS EMPLOYED: Hamburger is being 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 will be separated in an attempt to isolate and identify the mutagenic components. Similar work is being done with other fried meats, fried eggs and beef extracts.

MAJOR FINDINGS AND PROPOSED COURSE: Hamburger: A series of extraction procedures have been developed which greatly increase the yield of extracted mutagen. The hamburger mutagen requires metabolic activation and reverts only those Salmonella strains which are reverted by frameshift mutagens. Studies on the kinetics of hamburger mutagen formation showed that the cooking temperature, rate of heat transfer and level of dehydration all affect the level of mutagenicity. Following a Japanese report which identified two imidazo quinolines (IQ and MeIQ) beef mutagens, studies were performed, using preparative TLC followed by HPLC, which showed that the majority of the mutagenic activity in an acetone extract of hamburger had the same position on the HPLC trace as did IQ. IQ has been synthesized and chemistry and mutagenicity studies have been initiated. Me-IQ will be synthesized in the near future.

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.

Additions of amino acids to the beef extraction procedure showed that cysteine, proline and tryptophan all led to enhanced mutagen formation. The mutagens formed will be isolated and characterized.

Frying of meats and fish under standard conditions resulted in mutagen formation. Different meats yielded different levels of mutagen; selected meats and eggs will be used for mutagen isolation and characterization.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
It is well accepted that mutagenic substances are normal components of the environment. In order to place human exposure levels of mutagens in their proper perspective, it is important to characterize the levels and types of exposure. This project, which also includes a survey of food intake in the U.S., will attempt to produce estimates of this mutagen exposure. A major aspect of the program is the study of cooking practices or food additives (or treatments) which will retard or inhibit the formation of mutagens during the cooking process and thereby lower human exposure to these mutagens.

PUBLICATIONS

1. J.S. Felton, S. Healy, D. Stuermer, C. Berry, H. Timourian, F.T. Hatch, M. Morris, and L.F. Bjeldanes. "Mutagens from the Cooking of Food. I. Improved Isolation and Characterization of Mutagenic Fractions from Cooked Ground Beef." Mutation Research 88, 33-44 (1981).

2. J.S. Felton, S.K. Healy, D.S. Orwig, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, L.F. Bjeldanes, and M. Morris. "Metabolism of Mutagenic Fractions from Cooked Ground Beef." Cancer Res. 21, 125 (1980).

3. C. Plumlee, L.F. Bjeldanes, F.T. Hatch. "Food Item Priority Assessment for Studies of Mutagen Production During Cooking." J. Am. Dietetic Assoc. (in press).

4. F.T. Hatch, J.S. Felton, S. Healy, D. Stuermer, P. Berry, and H. Timourian. "Formation of Mutagens During Cooking of Protein Foods." J. Supramol. Struct. Cellular Biochem. Suppl. 5 (in press).

5. J.S. Felton, S.K. Healy, M. Knize, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, L.F. Bjeldanes, and M. Morris. "Metabolism of Mutagenic Fractions from Cooked Beef." J. Supramol. Struct. Cellular Biochem. Suppl. 5 (in press).

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: $50,875

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 in use.

METHODS EMPLOYED: Test procedures using Drosophila melanogaster will be developed for use in a test for aneuploidy induced by industrial and environmental chemicals. The development of the procedures will be accomplished as follows. The contractor will determine the most advantageous endpoints to use in a rapid screen of chemicals. The determination will be made after considering such factors as (a) ease of scoring, (b) relative merits of testing somatic or gonial events, and (c) whether secondary end-points should be monitored as well. The contractor will then determine the best procedure for monitoring the chosen endpoints. Variables that need to be determined include (a) gender of the treated animal, (b) the time during development which is best for treatment, (c) means of administration, (d) sample size and (e) positive control substances. After a protocol has been determined it will be validated by screening a number of selected coded chemicals.

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 Environmental Mutagenesis Test Development Program. 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 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-developed screen to detect such chemicals on a large scale.

LABORATORY OF PHARMACOLOGY

LABORATORY OF PHARMACOLOGY
Summary Statement

The Laboratory of Pharmacology carries out research to elucidate the relationships between the transformation and translocation of chemicals and toxicity in various target organs and cells of the body. A multidisciplinary approach is used in these investigations: pharmacologists, biochemists, chemists, endocrinologists, pathologists, physiologists, toxicologists, mathematicians and statisticians participate. This laboratory provides a central focus at NIEHS for using pharmacological and pharmacokinetic concepts to characterize, in detail, the mechanisms by which environmental contaminants exert biological effects. It plans and conducts studies 1) to determine the metabolic basis for selective/specific damage to certain organs and cell types which is characteristic of some toxins, 2) to elucidate the mechanisms whereby chemicals with hormonal activity alter the normal functions of organ systems, 3) to determine the role of membrane structure and function in excretion and toxicity of pollutants, 4) to correlate organ and cell structure with function both before and after exposure to chemicals, 5) to identify sensitive biochemical, pharmacological, physiological and pathological indicators of target organ/cell toxicity useful for the early detection and prediction of toxicity in experimental animals and humans, and 6) to provide reliable end points for the extrapolation of dose-dependent chemical effects across animal species and to humans. The Laboratory of Pharmacology also serves as a focal point within NIEHS, NIH and DHHS for marine and freshwater biomedical research. In this context we are especially interested in possible direct impact on human health by contaminants present in the aquatic environment (including drinking water) and accumulated by aquatic animals. Presently, the Laboratory of Pharmacology contains the following groups: Toxication-Detoxication Mechanisms, Comparative Pharmacology and Physiology, Pharmacokinetics, Receptor Pharmacology, Heavy Metal Toxicity and Target Organ Toxicity-Gastrointestinal Tract. Toxication-Detoxication Mechanisms and Comparative Pharmacology are each comprised of several tenured scientists whereas the other groups contain only one tenured scientist each.

Background

The Laboratory of Pharmacology was an original component of the NIEHS Intramural Research Program (IRP). Last year it consisted of two sections (Toxication-Detoxication Mechanisms and Comparative Pharmacology and Physiology) and four senior (tenured) scientists (Drs. Bend, Fouts, Philpot and Pritchard) and their support personnel. A reorganization occurred within the IRP late in FY '80, and as a result four additional tenured scientists and their subordinates joined the Laboratory of Pharmacology. These included Dr. Marshall Anderson (from the Laboratory of Pharmacokinetics) and Drs. George Lucier, Bruce Fowler and Carol Schiller (all from the Laboratory of Organ Function and Toxicology).

A. Toxication-Detoxication Group

The overall activity of this group can be described as an integrated, multifaceted effort concerned with understanding the role of chemical metabolism in the mediation of toxicity such as overt tissue damage, or more subtle effects such as carcinogenesis, mutagenesis, teratogenesis.

For many chemicals, the processes of metabolism are means of both activation and inactivation and the relative activities of these pathways/steps, as well as their location in different cells, and parts of cells, are most critical to the particular outcome of exposure to any given chemical. That these processes of metabolic activation and inactivation are themselves often controlled by genetics, as well as being affected by age, sex, disease and environment, further complicates the understanding of their role in the effects of any given chemical in any given tissue or animal species or individual of that species at any specific time of exposure.

Many different approaches to the study and use of chemical-metabolizing systems are being made in this and other laboratories both here at NIEHS and around the world. 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.

In Dr. Bend's laboratory, the focus is on a comparison of chemical metabolism in systems having various organizational states -- e.g., whole animal, perfused organ, isolated cellular components, and purified isolated enzymes and components of enzyme systems; detailed studies of particular, critical metabolic pathways and patterns -- e.g., stereo- and regioselectivity in conjugation of epoxides with glutathione; and tissue and species differences in selected, critical metabolic pathways -- e.g., benzo(a)pyrene metabolite patterns (both oxidative and conjugated products).

Dr. Philpot's workgroup concentrates on isolation and characterization of purified components of the monooxygenase system in lung versus liver, especially the cytochrome P-450 components and on the use of such purified components in better understanding the role of these systems in such things as target organ toxicity, species differences in toxicity, and localization of such systems in particular organs, cells, and parts of cells.

In Dr. Fout's laboratory, the focus is on chemical-metabolizing systems in liver, lung, and skin, on the localization of systems within specific cell types, on factors which affect these systems, on the development of these systems in the perinatal period, and on species differences in these systems.

Recent Accomplishments:

1. Dr. Bend's laboratory:

   a. Several metabolic factors have been elucidated which contribute to the selective pulmonary toxicity of certain polycyclic aromatic hydrocarbons (PAH).

   b. A method has been developed for studying the relationships between xenobiotic metabolism and the destruction of pulmonary enzymes such as cytochrome P-450. A perfusion technique is used, with one lobe of the lung serving as a test system and the other lobe serving as a control.

   c. We have begun to study the stereoselectivity of hepatic glutathione transferases with model alkene (styrene 7,8-oxide) and arene oxide

(benzo[a]pyrene 4,5-oxide) substrates. This work is being done in collaboration with the Laboratory of Environmental Chemistry.

2. Dr. Philpot's laboratory:

   a. A form of cytochrome P-450 found in lung activates certain promutagens much more rapidly than do the known hepatic forms of the enzyme.

   b. The lung toxin, 4-ipomeanol, is activated by two forms of cytochrome P-450 that are highly concentrated in the cell type that is the "target" for this chemical.

   c. NADPH-cytochrome P-450 reductases from liver and lung are the same enzyme as determined by spectral, catalytic, kinetic, immunochemical and structural characterization.

   d. Cytochrome b_5 participates in some NADPH-mediated reactions to a much greater extent in lung than in liver.

3. Dr. Fouts' laboratory:

   a. Lung research

      Xenobiotic metabolizing systems of type II and Clara cells of rabbit lung and from control vs. polycyclic hydrocarbon-induced (βNF) rat lung were studied. In both species the quantity and type of enzymes differ markedly in type II vs. Clara cells, which may explain specific cell damage by chemicals -- e.g., ipomeanol damage to Clara cells. Selected pathways (e.g., 7-EC dealkylation) can now be quantitated in single isolated rabbit lung cells and in rat Clara cells.

   b. Skin research

      Skin cells have been isolated and separated into fractions enriched in basal and sebaceous cells and some pathways for xenobiotic metabolism have been studied in cells from control vs. βNF-treated mice. Sebaceous cells were shown to be especially rich in certain metabolizing systems whereas basal cells are not; this agrees with ipomeanol covalent binding as seen in radioautographs. Rat Zymbal's gland is predominantly sebaceous cells and is rich in metabolizing systems. It is also a site where several chemical carcinogens cause tumors.

B. Comparative Pharmacology and Physiology Group

This group has programs in North Carolina (at NIEHS), at the C. V. Whitney Marine Laboratory for Experimental Biology and Medicine, University of Florida (Gainesville), Marineland, Florida, and at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine (summer season only). Dr. Bend heads the programs at NIEHS and in Maine and Dr. Pritchard heads the off-site marine biomedical laboratory in Florida. This group uses a variety of aquatic animals and mammalian species, for comparison purposes, to understand toxicological/pharmacological/physiological effects and problems. Major emphasis is currently focused on toxication-detoxication systems, transport and excretory mechanisms and membrane toxicity.

The uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism in the storage and the chemical form of the accumulated xenobiotics in these species is assessed. The major emphasis is on how, why and where marine species accumulate pollutants which have potential for harm to man and whether or not mixtures of pollutants may lead to accumulation of more toxic forms of higher levels of pollutants than single chemical exposure. Effect of water temperature on metabolic storage, and excretion processes is being studied. Particular emphasis is being placed on studying formation and further metabolism of chemically reactive metabolites (e.g., arene oxides from polycyclic hydrocarbons). Special importance is also given to the cytochrome P-450-containing monooxygenase (MO) system and other hydrolytic and conjugating pathways, including effects of pre-exposure to environmental contaminants on these enzyme systems. Where biologically significant enzyme induction is observed, the induced system is characterized in considerable detail.

The factors which determine the rate of xenobiotic excretion are also evaluated in aquatic and mammalian species in detail to help assess the mechanisms leading to toxicity of chemicals that occur as environmental pollutants.

Another major purpose of this section is to serve as a national focus for an aquatic pharmacology/toxicology program -- to promote awareness of and use of such aquatic species and systems in better understanding human disease and contributions of pollution to such disease.

Recent Accomplishments:

1. Dr. Pritchard's laboratory (Florida)

   a. Both brush border (BBM) (i.e., luminal) and basal-lateral membranes (BLM) have been purified in good yield from teleost kidney. In addition, the glucose carrier protein has been solubilized from BBM and reconstituted into proteoliposomes — the necessary first steps in isolation and purification of the carrier. Secretory sulfate transport has been characterized in the intact fish, the isolated tubule and the BLM vesicle. These preparations provide the basis for a systematic evaluation of the effects of foreign compounds on membrane transport.

   b. The chemical characteristics of polycyclic aromatic hydrocarbon metabolites play a critical role in their retention and toxicity to fish. For example, the 7,8-dihydrodiol of benzo(a)pyrene (BP-7,8-diol) shows net secretion *in vivo* and is eliminated more than 10 times more rapidly than the closely related BP-7-phenol. The rapid excretion of BP-7,8-dihydrodiol is mediated by the organic anion transport system and is, therefore, subject to inhibition by anionic xenobiotics including the phenoxyacetic acid herbicides.

2. Dr. Bend's laboratory (at NIEHS and in Maine)

   a. A glutathione transferase was isolated from a marine elasmobranch (the little skate, *Raja erinacea*) and purified to homogeneity because of its efficiency at converting benzo(a)pyrene 4,5-oxide (BPO) to glutathione conjugates. The enzyme has been partially characterized, including its stereo- and regioselectivity in the catalysis of the reaction between BPO and glutathione.

b. The wide variation of certain hepatic monooxygenase activities observed in winter flounder (Pseudopleuronectes americanus) and other teleost fish from Maine and Florida is apparently due to polycyclic aromatic hydrocarbon-like induction from environmental exposure to natural (certain flavonoids) or exogenous (PAH, dioxins, certain PCB or PBB isomers) xenobiotics. Those wild flounder with elevated aryl hydrocarbon hydroxylase activities were found to contain the same form of hepatic cytochrome P-450 that is induced by pretreatment with PAH.

c. The greater thermolability of NADPH-cytochrome P-450 reductase from Maine fish (vs. mammals) appears to be responsible for the lower thermostability of monooxygenase activities in the aquatic species. Preliminary experiments suggest that the flavin of the little skate reductase enzyme has an abnormal structure (relative to mammalian P-450 reductase) (Drs. Fouts and Philpot).

C. Pharmacokinetics Group

This group has traditionally been concerned with defining and measuring reliable dose-response relationships in animals which will provide a basis for estimating the magnitude of risk at specified levels of toxicant exposure. Toxicological data obtained at high doses can be extrapolated to low dose levels by means of various statistical approximations. These statistical models, however, often do not take into account the actual course of the multiple and often dose-dependent events governing the interaction of the chemical and its metabolites within the animal. A fundamental problem and the primary objective of this group is to characterize the most appropriate pharmacokinetic transformations of applied dose for use in the statistical models. For carcinogens such as polycyclic aromatic hydrocarbons, it is thought that specific DNA adducts are more important than total DNA binding in the initiation of carcinogenesis. Thus, it is appropriate that the amount of these specific adducts be the end point of the pharmacokinetic study. The amount of DNA adduct formed could be viewed as the "effective dose" for induction of tumor. This "effective dose" can be incorporated into low dose risk estimation models.

This program has merged into the Laboratory of Pharmacology during the past year and has made some readjustments so that its research goals will integrate more closely with those of pharmacology. It is investigating a very interesting concept -- pharmacokinetic considerations of the formation and repair of DNA adducts in target vs. non-target organs for carcinogenesis (in susceptible vs. nonsusceptible animal species). Thus, these scientists are concerned with relationships between metabolism (both activation and detoxication) of precarcinogens and the amount and chemical species of the adduct formed in various tissues as a result of dose, time, etc. Mechanistic aspects of the metabolic processes are to be closely correlated with DNA binding profiles. Compatible with these goals is examination of the mechanism(s) by which various inhibitors of carcinogenesis inhibit carcinogen-DNA adduct formation.

Recent Accomplishments

a. Advances have been made in understanding the mechanism(s) by which the antioxidant butylated hydroxyanisole (BHA) and aryl hydrocarbon hydroxylase (AHH) inducers inhibit the induction of tumors by benzo(a)-pyrene (BP). AHH inducers and BHA inhibit the formation of BP diol

epoxide-DNA adducts and the degree of inhibition correlates with the inhibition of tumorigenesis. Thus, AHH inducers and BHA appear to inhibit BP-induced neoplasia by inhibiting the formation of BP metabolite-DNA adducts. Our data are consistent with the hypothesis that AHH induction is a protective mechanism against polycyclic aromatic hydrocarbon-induced neoplasia.

b. Tissue differences in susceptibility to BP-induced neoplasia cannot be explained by differences in BP-DNA adduct levels or in vivo disappearance rates of the adducts when measurements are based on total DNA content of the organ. However, the data do suggest that determination of adduct levels in individual cell types in lung and liver might help explain the difference in the susceptibility of these organs to BP-induced neoplasia.

c. The predominant BP metabolite-DNA adduct formed in liver and lung of the male Sprague-Dawley rat was not a BP diol epoxide (BPDE) adduct but rather a BP-phenol-oxide adduct. This is the only known example where a bay region diol epoxide adduct is not the predominant, and in many cases the only, type of DNA adduct observed after in vivo administration of BP. The lack of formation of BPDE adducts in rat lung is consistent with the lack of induction of pulmonary tumors in rat by BP.

D. Receptor Pharmacology Group

The research of this group is concerned with various aspects of hepatotoxicity emphasizing the more subtle alterations in liver function following exposure to environmental agents. The major focus is to characterize the role of endocrine action in the regulation of hepatic function in control and pollutant-treated animals, including the role of hormone receptors and toxicant-receptor interactions.

Certain organs such as the uterus, vagina and the mammary gland are stimulated by estrogen. The presence of a receptor is presumed obligatory to a direct estrogenic effect on cellular machinery. Estrogen has been shown to modulate various aspects of mammalian liver function. Moreover, adverse side effects of estrogenic components of oral contraceptives on the liver are receiving increasing attention. Certain of these estrogen-induced changes could reflect a direct liver-hormone interaction dependent upon the presence of estrogen receptors in the liver cells. Studies demonstrate that liver cytosol fractions from both male and female rats contain estrogen-binding components possessing criteria assigned to receptor proteins. These criteria include a finite binding capacity together with a high affinity and binding specificity for estrogens. Two conformational forms of estrogen-receptor complexes are evident in both male and female liver nuclei. Interestingly, male liver contains an additional estrogen-binding protein distinct from the receptor. The physiological significance of this protein is under investigation. These binding proteins appear to be under pituitary-hypothalamic control. The presence of receptors indicates that the liver is a target organ for estrogens and the study of hepatic estrogen-receptor interactions and the consequences of this estrogen action is clearly of importance in determining the impact of estrogenically-active chemicals on liver function. The mycotoxin, Zearanol, has been shown to bind hepatic estrogen receptor as well as β-estradiol. Additionally, studies are in progress to ascertain the role of metabolism in the generation of estrogenic metabolites of PCBs and DDT derivatives. The ability of

both endogenous estrogens and estrogenically active xenobiotics to alter liver function and biochemistry is evaluated in isolated hepatocytes and isolated perfused liver. The goal of these studies is to investigate the relationship of hepatic estrogen action to various forms of organ-specific toxicity including cardiovascular disease, hypertension and hepatotoxicity.

Recent Accomplishments

a. Using the rat as an experimental model it was shown that the liver contains estrogen receptors that 1) have high affinity for estrogens, 2) are selective for estrogens, 3) have a finite binding capacity, 4) undergo nuclear translocation and bind tightly to chromatin, and 5) elicit specific biochemical responses. These studies have demonstrated that the levels of liver estrogen receptor play a rate-limiting role in estrogen-induced production of very low density lipoproteins as well as estrogen-induced modulation of serum enzyme proteins and liver cytosolic protein synthesis as evaluated by 2-D gel electrophoresis. We have also shown that the pituitary, in concert with sex steroids, regulates the responsiveness of the liver to estrogens by regulating levels of hepatic estrogen receptors.

b. In addition to directly affecting hepatic function, hormones might also regulate hepatic responsiveness to other xenobiotics. Sex differentiation of hepatic metabolism (including the drug-metabolizing enzymes) appears to be imprinted at birth by neonatal hormones during a narrow critical developmental state. Therefore, alterations in the hormonal milieu during this critical period could irreversibly change the susceptibility of the liver to hepatotoxins. Our studies have partially characterized the nature of pituitary regulation of specific forms of hepatic cytochrome P-450 and elucidated some of the age-specific endocrine parameters that ultimately regulate the levels and types of key metabolic activation/deactivation enzyme systems in liver.

E. Heavy Metal Toxicity Group

Principal efforts are oriented towards investigation of the mechanisms by which heavy metals produce subcellular damage using quantitative electron microscopy, biochemical, and physiological measurements. Particular emphasis is placed on understanding the mechanisms by which heavy metals interact with biological processes to effect toxicity in human populations. Specific studies include investigations concerning the mechanisms by which agents such as cadmium produce renal damage and low molecular weight proteinurias.

Another aspect of this work involves combined use of ultrastructural/morphometric and biochemical techniques to identify early effects of heavy metals on mammalian tissues so that tests for predicting subtoxic human responses to such agents may be developed. These studies include determining the correlation between quantitative subcellular morphologic observations and biochemical alterations in tissues of animals chronically exposed to low levels of toxic metals for various periods. Experimental results indicate that specific morphological and biochemical response profiles can be established which differentiate various metals in our multi-element environment.

Recent Accomplishments

a. Studies from this group have defined quantitative relationships between mitochondrial/cellular toxicity and urinary excretion of porphyrins. Arsenic, lead and methyl mercury each produce a distinctive spectrum of mitochondrial effects which are associated with specific urinary excretion patterns related to inhibition of mitochondrial heme biosynthetic enzymes in target tissues. Pb x Cd x As interaction studies have shown specific urinary porphyrin profiles for each combination of metals enabling assessment of toxicity in multi-element exposure situations.

b. An *in vivo* model for producing cadmium-induced tubular proteinuria via injection of cadmium-thionein was developed. This metal-protein complex is readily filtered by the glomerulus and reabsorbed by cells of the proximal convoluted tubules with rapid development of low molecular weight proteinuria as assessed by SDS-gel electrophoresis and isoelectric focusing. Ultrastructural morphometric/biochemical evaluation of proximal tubule cells indicated that the underlying mechanism involved decreased secondary lysosome formation with resultant inhibition of lysosomal protease activity.

c. An apparently inducible low molecular weight cadmium-binding protein, chemically distinct from mammalian metallothionein, was isolated from the American oyster and purified. This protein appears to be responsible for the rapid bioaccumulation of Cd by oysters and other edible marine molluscs. Circular dichroism studies done in collaboration with Dr. Chignell, Laboratory of Environmental Biophysics, indicate that while the oyster protein has a conformation distinct from metallothionein, a Cd-S bond signal is present in both proteins indicative of cysteine participation at the binding site(s).

d. Recent studies from this group demonstrated the presence of two previously unreported cytosolic Pb-binding components in kidney and brain, but not liver and lung of control rats. The binding components were found to be preformed constituents of kidney and brain cytosols and appear to be the initial primary ligands for Pb in these tissues.

F. Target Organ Toxicity-Gastrointestinal Tract

This group is developing approaches to study problems of intestinal toxicology and function at the cellular, subcellular and molecular level. Of particular interest are areas that represent unknowns in the field of intestinal absorption and metabolism that may be related to selective/specific gut toxicoses. 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.

The assimilation of dietary and endogenous lipid is central to the functions which maintain cellular integrity and biochemical processes and is an important focus for the toxic effects of lipophilic toxins. This group is interested primarily in the interactions of lipophilic toxins such as TCDD, with the intestinal

absorption of lipid and the mobilization and utilization of endogenous lipid as a basis for an understanding of the toxicity of these compounds as well as a probe to disrupt the normal intestinal absorptive processes. They will focus on lipid metabolism because 1) it is essential to the general metabolism and biomembrane structure of each organ, 2) lipid malabsorption may alter absorption and utilization of other nutrients, and 3) there is current evidence to suggest that lipid metabolism is altered by TCDD-like toxins.

Recent Accomplishments

a. L-Glycerol-3-phosphate dehydrogenase (G-3-PD) isozymes were partially purified from rat liver, colon and 1,2-dimethyl hydrazine-treated colon tumor cytosol. The antioxidants BHA and BHT markedly inhibited (40%) G-3-PD activity at 0.05 mM concentration, suggesting that this enzyme, as well as alcohol dehydrogenase, may be involved in generation of the ultimate carcinogen from methylazoxymethanol, and the inhibition of this reaction by BHA and BHT.

b. An HPLC method was developed for measuring UDP-GA levels. Concentrations of this co-factor for UDP-glucuronosyl transferase may reflect changes in the redox state and affect changes in the ability of cells to glucuronidate toxic xenobiotic metabolites.

c. Isolated intestinal cells are being used to examine protein synthesis and its regulation. The administration of TCDD (80 µg/kg by gavage) to rats, which were then fed corn oil, inhibited leucine incorporation in duodenal cells by approximately 50%. This data may, in part, explain the mechanism of starvation associated with TCDD treatment.

G. Collaborative Efforts

As can be seen from the individual project descriptions, scientists in the Laboratory of Pharmacology are involved in many activities and collaborative research efforts with scientists here at NIEHS and elsewhere. Especially noteworthy are the interactions at our marine laboratories in Maine and Florida where a wide variety of interdisciplinary research is carried out with a large number of scientists in residence at these laboratories.

Examples of collaborative programs outside of NIEHS for each of the senior scientists are: Dr. Bend with Dr. Bengt Mannervik of the University of Stockholm, Dr. David Peakall of the Wildlife Toxicology Division of the Canadian Wildlife Service and Drs. Don Reed and Mike Meredith of Oregon State University; Dr. Fouts with Drs. Mike Boyd and Charles Statham, National Cancer Institute, Dr. Ping Pan, U. S. Department of the Interior, Dr. Leakey at the University of Dundee and also with Dr. Meredith and Reed, Oregon State University; Dr. Lucier with Dr. Kern of the University of Colorado Medical Center; Dr. Philpot with Dr. Eric Johnson of Scripps Clinic and Research Foundation, Dr. Paul Thomas of the Hoffman-LaRoche Institute of Molecular Biology and Dr. Boyd of NCI; Dr. Pritchard with Dr. David Miller, Mount Desert Island Biological Laboratory; Dr. Anderson with Dr. Bob Dedrick, Chemical Engineering Section, NIH and Dr. Jim Selkirk, Oak Ridge National Laboratory; Dr. Fowler with Dr. David Engel, NOAA, Department of Commerce, Dr. Kathryn Mahaffey of the Food and Drug Administration and Dr. Ian Armitage, Yale University; and Dr. Schiller with Dr. Boris Yagen of Hebrew University, Israel.

The collaborative efforts are cited only to show the extensive interactions of this Laboratory with groups outside NIEHS. In addition to these contacts, those with faculty and researchers in the Triangle area are too numerous to document, but add strength to our activities, peer reviews (in terms of seminars, discussions, exchange of students), and opportunities for advice, new techniques, and short courses not only for our staff but for members of the other institutions as well.

H. Personnel

Dr. J. R. Bend became Chief of the Laboratory of Pharmacology in December, 1980. New additions to the laboratory during FY '81 were Ms. Debbie Garner (office), Dr. Richard Rumbaugh (Staff Fellow with Dr. Lucier), Dr. Mahmooda Kulkarni (Senior Staff Fellow with Dr. Anderson), Ms. Marian Frech (Technician with Dr. Pritchard in Florida), Ms. Rebecca Sparks (Technician with Dr. Fouts), Ms. Carol Bixler ("P" appointment with Dr. Anderson) and Mr. Craig Harris ("P" appointment with Dr. Bend). Four scientists that are supported by NIH Post-doctoral Fellowships entered the laboratory this year (Dr. Margaret Miller with Dr. Lucier, Dr. David Petering and Dr. Winona Victory with Dr. Fowler, and Dr. Chon Shoaf with Dr. Schiller). Temporary employees this year were Ms. Gail Fulton (office), Mr. John Holland (Technician with Dr. Fouts) and Ms. Scarlett Raeford (Technician with Dr. Lucier).

Those leaving the Laboratory of Pharmacology were Dr. Louise Ball, Dr. Coral Lamartiniere, Dr. Agneta Oskarsson, Dr. Brian Smith, Dr. Yu Ying Liu, and Ms. P. Clawson.

I. 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; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee and member, Scientific Advisory Committee, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; Associate Editor, Biological Basis of Detoxication. Invited participant at a workshop on Drug Metabolism and Toxicity (sponsored by ASPET), Houston, Oct. 9-12, 1980; at the 11th International Meeting of the Princess Takamatsu Cancer Research Fund, Tokyo, Nov. 11-13, 1981; at an international symposium on "Organophosphates and the Marine Environment," Duke University Marine Laboratory June 8-10, 1981; presented seminars at the Mount Desert Island Biological Laboratory, The Zoological Institute, University of Tokyo and the Department of Pharmacology, Keio University School of Medicine, Tokyo; NIEHS Coordinator for "Special Topics in Toxicology" a graduate course given for the third consecutive year in Research Triangle Park for students at Duke, North Carolina State and University of North Carolina; served on graduate student committees at North Carolina State University and University of North Carolina.

Dr. J. R. Fouts: Adjunct Professor of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill; Adjunct Professor of Toxicology, Department of Entomology, School of Life Sciences, North Carolina State University at Raleigh; member, Toxicology Advisory Committee, Faculty of Toxicology, North Carolina State University; member, Epilepsy Advisory Committee, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethedsa; counselor, American Society for Pharmacology and Experimental Therapeutics; editorial boards and editorial duties for many pharmacology journals. Lectures to medical students and graduate students at UNC -e.g.,- three lectures in Pharmacology 206, Biotransformation of xenobiotics and discussion panels. Served on graduate student committee - e.g. - Lori Dostal, Department of Pharmacology, UNC and Jeffrey Boyd, Toxicology Curriculum, N.C. Seminars at UNC and at N.C. State "Metabolism of Drugs in Isolated Lung Cells."

Dr. G. W. Lucier: Adjunct Associate Professor, Department of Biochemistry and Nutrition and Curriculum in Toxicology, University of North Carolina, Chapel Hill; Provisional member of Graduate School Faculty, University of North Carolina School of Medicine; Co-editor Environmental Health Perspectives; Editorial Boards of Peadiatric Pharmacology and Journal of Applied Biochemistry; Invited participant/ speaker to Institute de la VIE Conference on Biology and Futute of Mankind, Cold Springs Harbor Symposium on the Response of the Developing Organism to environmental agents and EPA conference on organ and species specificity in chemical carcinogenesis; Chairman, session on endocrine pharmacology at 1981 FASEB meeting and presented overview of session. Invited lecturer to Brigham Young University, University of Colorado Medical Center, and National Center for Toxicological Research; Consultant to EPA on implementation of the Toxic Substances Act as it applies to children.

Dr. R. M. Philpot: Adjunct Associate Professor, Department of Entomology, North Carolina State University, Raleigh; member, Toxicology Advisory Committee, North Carolina State University, Associate Managing Editor (U.S.A.), Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; member, Editorial Board, Molecular Pharmacology. Invited speaker at the symposium on the Biochemistry, Biophysics and Regulation of Cytochrome P-450 held in Stockholm, Sweden and presented seminars and course lectures at the University of California at Los Angeles, The University of Utah, The Uniformed Services University of Health Sciences and before the Genetic Toxicology Association.

Dr. M. W. Anderson: Adjunct Associate Professor, School of Medicine, Duke University; member of the Committee on Pyrene and Selected Analogs, National Research Council, National Academy of Sciences; Invited speaker at the National Conference on "Structure Activity Correlations as a Predicitve Tool in Toxicology," Research Triangle Park, N.C.; and at a symposium on "The Non-respiratory Metabolic Functions of the Lung," FASEB, 1981.

Dr. B. A. Fowler: Adjunct Associate Professor, Department of Pathology, University of North Carolina, Chapel Hill; member, editorial board, Chemico-Biological Interactions; Invited participant at the Symposium on Scientific Basis of Toxicity Assessment; Symposium on Micronutrient Interaction at the New York Academy of Sciences; Symposium on Nephrotoxic Mechanisms: Drugs and Environmental Toxins; U.S.-Japan Workshop on Metallothionein, International Workshop on Metals and Carcinogenesis; DOE/NBS Workshop on Environmental Speciation and Monitoring for Trace Metal-Containing Substances from Energy-Related Processes. Invited to

present Seminars at Mt. Sinai School of Medicine, Argonne National Laboratory, New York University.

Dr. J. B. Pritchard: Adjunct Associate Professor, Department of Pharmacology, University of Florida School of Medicine, Gainesville.

Dr. C. M. Schiller: Adjunct Associate Professor, Department of Biochemistry and Nutrition, School of Medicine, University of North Carolina, Chapel Hill; member of the Faculty of the Graduate Curriculum in Toxicology, University of North Carolina, Chapel Hill; Liaison Member, U.S.-EPA Toxic Substances Subcommittee, Science Advisory Board, Washington, D.C.; member, Digestive Diseases Coordinating Committee, Bethesda, MD; Alternate member, Nutrition Coordinating Committee, Bethedsa, MD; Lecturer in graduate-level courses in Biochemical Toxicology at the University of North Carolina, Chapel Hill and at Duke University. Sponser of graduate students from the Department of Biochemistry and Nutrition and the Curriculum in Toxicology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35005-02 LP*

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Considerations in the Formation and Repair of Carcinogen-DNA Adducts

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

PI:	Marshall W. Anderson	Mathematician	LP	NIEHS
Other:	Dr. Peter I. Adriaenssens	Visiting Fellow	LP	NIEHS
	Dr. Mahmooda Kulkarni	Senior Staff Fellow	LP	NIEHS
	Ms. Carol Bixler	"P" Appointment	LP	NIEHS
	Dr. Robert M. Pratt	Research Chemist	LRDT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Reproductive and Developmental Toxicology

LAB/BRANCH

Laboratory of Pharmacology

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.9	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

There is much evidence to suggest that the extent of carcinogen-induced promutagenic DNA damage and the capacity of cells to repair such damage represent critical events in the initiation of carcinogenesis. We are studying the in vivo formation and repair of benzo(a)pyrene metabolite-DNA adducts in target and non-target organs for benzo(a)pyrene-induced neoplasia. We are concerned with the effect of dose of BP and inhibitors of benzo(a)pyrene-induced carcinogenesis on the amount and type of adducts formed. Emphasis is on studies which enhance our understanding of the relationship between metabolism of BP and the amount and types of DNA adducts formed in the various tissues.

*This is a combination of projects Z01 ES 35003 LPK, Z01 ES 35005 LPK and Z01 ES 35006 LPK.

PROJECT DESCRIPTION

OBJECTIVES: 1) To further test the hypothesis that the formation of bay region diol epoxides-DNA adducts is a necessary step for tumor initiation by benzo(a)-pyrene (BP). 2) To test the hypothesis that the extent of BP metabolite-DNA adduct formation and/or repair of such damage can explain the difference in organ and species susceptibility to BP-induced neoplasia. 3) To examine the effect of the initial amount of BP metabolite-DNA adduct formed on the enzymatic repair of the adducts in a target and non-target organ for BP-induced neoplasia. 4) 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. 5) To examine the relationship between the metabolism of BP and the amount and types of BP metabolite-DNA adducts formed in the various tissues. 6) 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: Animals were treated with various doses of ^{3}H-BP and then sacrificed at various time points. DNA was isolated from tissue by either the classical phenolic extraction procedure or 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.

MAJOR FINDINGS AND PROPOSED COURSE: 1. A DNA isolation procedure based on a phenolic extraction followed by hydroxyapatite chromatography was developed. This procedure has several advantages over the classical phenolic extraction procedure in which numerous extractions, precipitations, and dissolutions are involved. The hydroxyapatite method is faster and gives better yields and clearer samples of DNA. We believe that most of the early eluting radioactive peaks observed in either HPLC or LH-20 chromatography from DNA isolated by the classical phenolic extraction procedure represent contamination by protein and/or RNA and are not a result of ^{3}H-BP-damaged DNA. However, the specific activities of the BP metabolite-DNA adducts were independent of the procedure used to isolate the DNA.

2. The data are consistent with the hypothesis that the formation of bay region diol epoxides-DNA adducts is a necessary step in tumor initiation by BP. After *in vivo* treatment of the animal with BP, examination of the tissues known to be susceptible to BP-induced neoplasia showed that 7β,8α-dihydroxy-9β,10α-7,8,9,10-tetrahydroxybenzo(a)pyrene (BPDEI)-deoxyguanosine was the predominant adduct observed. The tissues examined include the lung and forestomach of the A/HeJ and ICR/Ha mice and the lung of rabbit. Dose regimens of BP that are known to induce neoplasia were used. The 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydroxybenzo(a)pyrene (BPDEII)-deoxyguanosine adduct and an unidentified adduct, probably a BP-phenol-oxide-DNA adduct or a BPDEI-deoxycytidine adduct, were also detected in these tissues (8-15% and 10-20% of the BPDEI-deoxyguanosine adduct, respectively). In contrast to the results obtained in the tissues of the mice and rabbit, the predominant BP metabolite-DNA adduct in the male Sprague-Dawley rat was not the BPDE adducts but rather a BP-phenol-oxide-DNA adduct, possibly resulting from the interaction of 9-OH-BP-4,5-oxide with DNA. The BPDE adducts were not detected in rat liver and only a small amount (3.3% of the BP-phenol-oxide adduct) was observed in rat lung. This is the only known example

where a bay region diol-epoxide adduct is not the predominant, and in most cases the only, type of DNA adduct observed after in vivo administration of a polycyclic aromatic hydrocarbon. The lack of formation of substantial amounts of BPDE adducts in rat lung is consistent with the lack of induction of pulmonary tumors in rat by BP.

3. The in vivo disappearance rates of BP metabolite-DNA adducts in lung and liver of A/HeJ mice were determined at two dose levels of BP. The oral dose of 6 mg per mouse is known to induce pulmonary adenomas and the other dose was 450-fold less than the carcinogenic dose. The amount of BPDEI-DNA adducts, as well as the other adducts, decreased monoexponentially with time at each dose. Moreover, even though the initial amount of the BPDEI adduct formed in lung and liver was approximately 1200 times less at the lower dose, the disappearance rates in lung and liver were very similar at the two dose levels of BP. The half-life for disappearance was 17 days in lung and 9 days in liver at the 6 mg per mouse dose and 19 days in lung and 16 days in liver at the lower dose. Thus, no dose threshold appears to exist for removal of BP metabolite-DNA adducts in either the target or non-target organs for BP-induced neoplasia in this mice strain. Similar studies will be done in rat and rabbit.

4. The lung and forestomach of A/HeJ mice are susceptible to BP-induced neoplasia whereas the liver is resistant to BP-induced neoplasia. Neither the initial amounts of adducts formed nor their disappearance rates differentiate between lung and liver and, therefore, do not appear to offer any explanation for susceptibility of the lung versus resistance of the liver to BP-induced neoplasis. The specific activities in our in vivo studies are calculated on the basis of the total DNA in the organ. It is likely that the amounts of adduct formed in different cell types vary considerably. This possibility has the greatest implications for organs, such as the lung, that contain a multitude of cell types. Although little is known about the localization of carcinogen-DNA adducts in lung, cytochrome-P-450-dependent monooxygenase enzymes appear to be much more localized in lung than in liver. Since the average values for the specific activities of the initial amount of BPDE-DNA formed are similar in lung and liver, these considerations suggest that adduct levels in certain pulmonary cell types might be much higher than those in hepatocytes. Our efforts are directed toward exploring this possibility. An in vivo-in vitro approach will be used in which specific activities of adduct are determined in isolated cell types after in vivo administration of BP. Autoradiography studies will also be pursued. Techniques exist in the Laboratory of Pharmacology for isolating cell types in lung of several species. The autoradiography studies will also assist us in pursuing a problem associated with the interpretation of in vivo disappearance rates of adducts as enzymatic repair of damaged DNA; e.g., the differentiation between enzymatic repair of carcinogen-DNA adducts and cell turnover since both processes lead to in vivo disappearance of adducts. For example, even though the disappearance rates of adducts are similar in lung and liver of A/HeJ mice, the actual enzymatic repair rates of the damaged DNA could be very different. This problem has been ignored in the literature and most investigators have equated in vivo disappearance rates of adducts with enzymatic repair.

5. Neither the specific activities based on total DNA content of the organ nor their in vivo disappearance rates explain the difference in susceptibility to BP-induced pulmonary neoplasia between the A/HeJ and C57BL/6J strain of mice.

Autoradiography studies as well as an in vivo-in vitro approach will be used in a further attempt to explain this strain difference in susceptibility to BP-induced neoplasia.

6. The effect of the antioxidant, butylated hydroxyanisole (BHA), on BP metabolite-DNA adduct formation has been examined under conditions known to result in inhibition of BP-induced neoplasis by BHA. In each tissue examined the decrease in the amount of BPDE-DNA adducts formed in the target tissue appears to correlate with the inhibition of BP-induced neoplasia by BHA. The mechanism(s) by which BHA treatment inhibits the formation of BPDE-DNA adducts is under investigation. The effect of BHA on BP metabolite-DNA adduct formation is being examined as a function of BP dose. All studies on the effect of BHA, as well as other antioxidants, on BP-induced neoplasia have been at relatively high dose levels of BP since bioassay studies are not feasible at the lower dosages. Because correlation between inhibition of BP metabolite-DNA adduct levels and BP-induced neoplasia existed at the observable carcinogenic dose, these studies should suggest what the prophylactic effect of BHA might be at levels of BP that are similar to those in the environment.

7. 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 animal examined. The effects of AHH inducers on the binding of BP to DNA in vivo contrasts with their effect in vivo. The mechanism(s) by which AHH inducers inhibit the in vivo formation of BPDE-DNA adducts is under investigation.

8. The correlation of mechanistic aspects of the metabolic processes with in vivo BP-DNA binding profiles is being investigated. The metabolite profiles of the glucuronide and sulfate conjugates present in the bile of rat are in general agreement with the BP-DNA adduct profiles. Changes in the nature and amount of BP-DNA adducts in the lungs, but not liver, of BHA-treated rats correlated with changes in biliary metabolite profiles. Future efforts will concentrate on the rabbit as the animal model system. The rabbit is a good model system for these studies as the cytochrome P-450 monooxygenase enzymes have been characterized in detail in lung and liver of the rabbit and the effects of various inducers of this enzyme system are documented. In addition, the rabbit is susceptible to polycyclic aromatic hydrocarbon-induced pulmonary neoplasia. These studies will be done in collaboration with Drs. Bend and Philpot.

9. Our results and others suggest that in vivo carcinogen-DNA levels in the target tissue can be used as the effective dose in the low-dose risk estimation of chemical carcinogens. Studies with inhibitors of BP-induced neoplasia show that tumor response changes quantitatively with BPDE-DNA adduct levels.

Similar correlations have been observed in other studies in which carcinogen-DNA adduct levels in the target tissue and tumor response have been measured simultaneously. Obviously, the specific activities of carcinogen-DNA adducts in the target cells of the target tissue would be a better measure of the effective dose of a carcinogen than the values based on total DNA content of the organ. However, this is probably not practical in most cases. Even if carcinogen-DNA levels and/or their removal rates calculated from total organ DNA do not explain organ and species difference to chemical-induced neoplasia, this should not distract from the ability of carcinogen-DNA adduct levels to predict low-dose response to carcinogens in known target tissues. This use of the amount of critical carcinogen-DNA adducts as a predictive tool in carcinogenesis is of practical significance since levels of adducts can usually be measured at dosages much lower than those used in bioassay studies. Dose-response curves for BP-DNA adducts will be determined in various target and non-target tissues in mice and rabbits.

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.

PUBLICATIONS

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Boroujerdi, M., Kung, H. C., Wilson, A. G. E., and Anderson, M. W.: Metabolism and DNA binding of benzo(a)pyrene in vivo in the rat. Cancer Res. 41: 951-957, 1981.

Anderson, M. W., Bouroujerdi, M., and Wilson, A. G. E.: Inhibition in vivo of the formation of adducts between metabolites of benzo(a)pyrene and DNA by butylated hydroxyanisole: Possible relationship to anticarcinogenic mechanisms. Cancer Res. 1981. In press.

Wilson, A. G. E., Kung, H. C., Boroujerdi, M., and Anderson, M. W.: Inhibition in vivo of the formation of adducts between metabolites of benzo(a)pyrene and DNA by aryl hydrocarbon hydroxylase inducers: Possible relationship to anticarcinogenic mechanisms. Cancer Res. 1981. In press.

Anderson, M. W., Boroujerdi, M., Wilson, A. G. E., Kung, H. C., and Ioannou, Y. M.: Formation in vivo of benzo(a)pyrene metabolite-DNA adducts in lung and liver of mice and rats. Fed. Proc. 1981. In press.

Anderson, M. W., Boroujerdi, M., Ioannou, Y. M., Kung, H. C., and Wilson, A. G. E.: Modifying factors affecting toxic responses: Example - inhibitors of carcinogenesis. Proceedings of the Conference on Structure Activity Correlation as a Predictive Tool in Toxicology. Hemisphere Publishing Co. 1981. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70120-09* LP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormonal Modulation of Hepatic Function

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.9	1.4	2.5

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

These studies are designed to assess the capacity of hormonally active chemicals to alter hepatic metabolism and to determine if changes in the endocrine environment can influence hepatic enzyme development and hepatotoxic responses. The rat is used as the experimental model. The initial focus has involved sex differentiation of microsomal hemoproteins in rat liver and the response of hepatic monooxygenase to cadmium; male liver monooxygenases activities are repressed by cadmium whereas female enzymes are unaffected by this treatment. Male sensitivity to cadmium appears to reflect the amount of cadmium-sensitive hemoproteins (detected on SDS gels and by hemoprotein synthesis/degradation studies) which are under hypophyseal control. Additionally, hepatic enzymes and specific forms of cytochrome P-450 are under varied forms of control by the hypophyseal-gonadal axis.

* Includes Z01 ES 70135-03 LOFT

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES AND METHODS EMPLOYED: I. Describe sex differentiation of hepatic enzymes including the hepatic monooxygenase system (HMOS) in the rat. This is accomplished by determining age-dependent differences in HMOS. Cytochrome P-450-dependent enzyme activities and kinetics are studied as well as hepatic hemoproteins (amount, synthesis/degradation, electrophoretic profile).

II. Investigate regulatory aspects of rat HMOS. These studies emphasize endocrine influences and the neonatal imprinting of liver biochemistry. Experiments to elucidate functional interactions of the pituitary-hypothalamic gonadal-hepatic axis include neonatal or adult castration followed by hormone replacement, hypophysectomy (immature or mature rats), use of ectopic pituitaries and adrenalectomy.

III. Determine if the imprinting of rat liver biochemistry can be altered by endocrine manipulations. The hormonal milieu is altered by: (1) administration of hormonally active chemicals, and (2) reduction in androgen levels at different developmental stages by methadone administration which effects a reversible castration.

IV. Investigate the role that sex differentiation of the HMOS plays in susceptibility to hepatotoxic agents in the rat. These studies use cadmium as hepatotoxic chemicals; cadmium alters HMOS in a sex-dependent manner. Endocrine influences described in Part II are used to understand the mechanisms responsible for this sex-dependent response. In addition to effects in HMOS, biochemical, physiological, and histological evaluations are made. The possible role of sex differences in pharmacokinetic parameters is also investigated, i.e., the role of metallothionein inducibility in the sex-dependent response of the liver to cadmium. The importance of the HMOS in sex differences (endocrine influences) in hepatocarcinogenicity will be investigated by altering the hormonal environment at different developmental stages and giving carcinogens later in life.

MAJOR FINDINGS AND PROPOSED COURSE: Sex-related differences in the hepatic microsomal ethylmorphine N-demethylation activities were observed only in control mature rats; specific activities in males were four-fold higher than in females. Administration of testosterone propionate (TP) or diethylstilbestrol (DES) to neonates resulted in the feminization (depression) of hepatic ethylmorphine N-demethylation activities of adult male rats without significantly altering the enzyme activities in either the mature female rats or immature rats of either sex. Furthermore, kinetic studies revealed neonatal feminization of Km values for the N-demethylation in adult male rats. Reduction in serum androgen levels of adult male rats was associated with the treatment of neonates with DES but not with TP. Reduction of hepatic ethylmorphine N-demethylation activity and cytochrome P-450 contents following cadmium treatment (2.0 mg/kg, ip) was also age- and sex-dependent in the rat; marked reduction in enzyme activity was observed only in adult male rats. Adult male rats which had received TP or DES treatments during the neonatal period exhibited decreased sensitivity towards the hepatotoxic effects of cadmium; responses in the treated groups were similar to those of females. The sex-dependent response to cadmium is under pituitary-hypothalamic control and is associated with the amount of cadmium-sensitive forms of cytochrome P-450 as demonstrated by synthesis/degradation studies on hepatic hemoproteins.

Analysis of hepatic hemoproteins on SDS gels have demonstrated that the levels of cadmium-sensitive forms appear to be imprinted by androgens at the pituitary-hypothalamic level during a critical period of neonatal development.

Administration of methadone to male neonates resulted in female type Km values for the hepatic monooxygenase system in the subsequent adult animal whereas V_{max} values were unchanged. This feminization of kinetic values appears to be related to a methadone-mediated depression of testosterone levels in neonates, suggesting that methadone prevents the imprinting of the monooxygenase system. This idea was reinforced by the finding that simultaneous administration of testosterone and methadone to neonates resulted in normal sex differentiation of enzyme activity and kinetic constants. Further studies demonstrated the critical neonatal period for the ability of methadone to feminize the HMOS.

We have selected other enzyme markers on the basis of metabolic function, organelle localization, and susceptibility to biochemical insult. These routinely measured enzyme systems are 1) glutathione S-transferases (cytosolic drug metabolism); 2) UDP-glucuronyltransferase (microsomal drug metabolism; 3) monoamine oxidase (mitochondrial biogenic amine metabolism); 4) histidase (cytosolic amino acid metabolism) and 5) cholinesterase (serum acylcholine hydrolysis). These enzyme markers are usually characterized by low catalytic activity during normal prepubertal life but activities increase in the adult rat resulting in enzyme levels which are significantly higher in one sex than the other. The first 2 have greater activity levels in adult males than females while the latter 3 are higher in adult female rats.

Glutathione S-transferases. No change in activity levels are observed following postpubertal gonadectomy of males or females. Testosterone propionate (TP) or estradiol-17β (E_2) administered to these gonadectomized animals also cause no change in activity; therefore androgen and estrogen are not direct modulators of the glutathione S-transferases. Neonatal castration of males, however, results in adult enzyme activities being feminized (decreased). Hypophysectomy results in elevated enzyme activities and a pituitary transplant under the kidney capsule reverses the effect of hypophysectomy thus suggesting that glutathione S-transferase activity levels are modulated by a pituitary inhibiting factor that is not dependent on a hypothalamic releasing factor(s). Reduced glutathione S-transferase activities in male rats treated with monosodium glutamate to induce arcuate nucleus lesions suggest that pituitary-dependent glutathione S-transferase may, however, be controlled by a hypothalamic inhibiting factor.

Two-dimensional Electrophoresis of Hepatic Proteins. We have developed a two-dimensional gel electrophoresis system to analyze ontogeny of hepatic proteins in normal animals and animals exposed perinatally to environmental chemicals. We have developed a system using isolated rat hepatocytes for the incorporation of high activity radioisotopes for visualization of protein patterns by autoradiography after electrophoresis. Using these methods we have been able to identify certain hepatic protein patterns that are characteristically male and others that are female type. Preliminary experiments indicate that the protein profiles can be quantitatively and qualitatively altered by changes in the neonatal endocrine environment.

In summary, our findings demonstrate that function of the HMOS may be irreversibly changed by alterations in the hormonal milieu during a critical period of early development. These alterations in HMOS components are correlated with hepatotoxic responses to xenobiotics such as cadmium.

Future studies will focus on the: (1) nature of the pituitary factors that regulate hepatic protein synthesis, (2) the mechanism of action of pituitary factors on liver enzymes (i.e., receptor interactions), or (3) the role of the peripheral endocrine systems on the regulation of hepatic enzymes, including androgen-responsive forms of cytochrome P-450.

The mechanisms of the sex-dependent response to the hepatotoxic actions of cadmium will be studied by: (1) evaluating metallthionein inducibility in male and female rats as a function of altered endocrine status, (2) sex differences in tissue distribution, (3) sex differences in hemoprotein electrophoretic profile, and (4) further studies on effects of hypophysectomy and gonadectomy on the HMOS response to cadmium.

Additionally, further studies will reflect objectives not yet accomplished; primarily Objectives IV and V. These investigations will emphasize the development of *in vitro* systems to assess hepatotoxicity including toxic actions of estrogenically active chemicals as discussed earlier. Morphological and functional parameters will be investigated in both *in vivo* and *in vitro* systems.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In addition to directly affecting hepatic function, hormones might also regulate hepatic responsiveness to other xenobiotics. Because some aspects of liver biochemistry and physiology undergo postpubertal sex differentiation, it might be expected that a corresponding differentiation could occur in the interactions of chemicals with liver cell components. Sex differentiation of hepatic metabolism is under pituitary-hypothalamic control and (including the drug-metabolizing enzymes) appears to be imprinted at birth by neonatal hormones during a narrow critical developmental stage. Therefore, alterations in the hormonal milieu during this critical period could irreversibly change the susceptibility of the liver to hepatotoxins. Previous studies have shown that neonatal estrogens can increase the incidence of chemically induced hepatocarcinoma (Weisburger *et al.*, Endocrinology, 82: 685, 1968). Since environmental chemicals may directly (receptor interactions) or indirectly (modification of metabolism and/or clearance of endogenous chemicals) elicit changes in hormone action, it becomes important to investigate the role of hormonally active chemicals in the generation of groups at risk to various forms of organ-specific toxicity.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70132-02 LP

PERIOD COVERED
October 1, 1980 To September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Intestinal Metabolism*

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

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	R. Yagen	Chemist	Hebrew Univ./Israel
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SUMMARY OF WORK (200 words or less - underline keywords)

Our focus is the development of approaches to study the regulation of gastrointestinal functions. Of particular concern are the regulation of intestinal absorption and metabolism, and the responses related to oral exposure to toxins. Current examples of these studies are the role of L-glycerol-3-phosphate in colon energy metabolism, NAD-linked L-glycerol-3-phosphate dehydrogenase in the metabolism of methylazoxymethanol and tumorgenesis, the factors involved in relative rates of intestinal tip and crypt cell protein synthesis, and the inhibition of lipid absorption and intestinal cell protein synthesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The regulation of these processes is examined necessarily at the cellular, subcellular and molecular levels.

*Formerly titled "Gastrointestinal Function and Toxicology"

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Many of the techniques employed have been derived from those devised with other tissues, especially liver, and are described in the bibliographic references. Techniques that have been devised by us to monitor intestinal processes in laboratory animals include: 1. separation and partial purification of liver and colon NAD-linked L-glycerol-3-phosphate dehydrogenase and alcohol dehydrogenase; 2. purification and characterization of cytosolic L-glycerol-3-phosphate dehydrogenase isozymes from liver, colon and tumor tissues; 3. preparation of monodispersed suspensions of viable colon cells; and 4. determination of acrylamide in body fluids.

Of special interest is the method we devised for separating NAD-linked L-glycerol-3-phosphate dehydrogenase from alcohol dehydrogenase. Our method involves the sequential affinity chromatography of liver or colon cytosol on Matrex Blue A and Matrex Red A. The L-glycerol-3-phosphate dehydrogenase is retained on the Matrex Red A and is then eluted with 1.0 mM NADH and the effluent is passed through Matrex Blue A and eluted with 0.150 mM $(NH_4)_2SO_4$. This method provides complete separation of the enzyme activities and sufficient purification for use in experiments to illustrate the relative rates of inhibition by antioxidants and of metabolism of the colon carcinogen methylazoxymethanol.

The chemical determination of acrylamide in the milk from lactating rats required extraction of the milk with methanol that contained Tris, followed by incubation with a diazomethane solution at 25°C. The diazomethane was generated for use with each experiment by the method of De Boer and Backer. The resultant complex was incubated with 4-dimethylaminocinnamaldehyde and measured spectrophotometrically at 538 nm. The time course of the appearance of acrylamide in the milk was used as part of a follow-up study to a cross-fostering experiment that indicated a lactational effect.

MAJOR FINDINGS: Colon-specific carcinogenesis -- The synthetic compounds 1,2-dimethyl-hydrazine and methylazoxymethanol, both chemically related to the naturally occurring carcinogen, cycasin, have proved to be of great value in their reliable and specific ability of produce colon tumors in several rodent species. With animal models, it becomes possible to study modifying influences on the initiation and development of colon cancer under strictly controlled laboratory conditions, and thus, to approach a better understanding of the etiology of tumor induction. In our laboratory, male Fischer rats were treated at 7 weeks of age with a single oral dose of 1,2-dimethylhydrazine (35 mg/kg). After 1.5 years, the 14 control and 28 treated animals were sacrificed for general autopsy and routine clinical analyses. The incidence of tumor formation in the treated animals was 78.6% as compared to 0% for the control animals. Routine hematologic evaluation and blood chemistry profiles were developed on the serum from nine control and ten treated animals. The significant differences observed were increases in the creatine phosphokinase and albumin/globulin values without any increase in total serum protein. Although the creatine phosphokinase levels in the treated animals were elevated markedly as compared to the control values, there was no difference in body weights between control and tumor-bearing animals. Of the many serum enzymes that may be elevated after acute myocardial infarction, only five, including

creatine phosphokinase, have withstood critical clinical and laboratory investigations of their value as indicators of heart damage. Although creatine phosphokinase serum levels peak early (33 h) after a myocardial infarction, they return to normal within 3 days. Creatine phosphokinase is apparently absent from liver in human and rat tissue but is present in both species in skeletal muscle. In the rat, there is also activity in the small intestine. Thus, this elevation of serum creatine phosphokinase levels may be the result of insult to one of these major organs.

A second group of animals given a similar oral dose is being evaluated in terms of creatine phosphokinase and lactate dehydrogenase isozymes in the serum, liver, colon, tumor (when present), brain, heart and skeletal muscle obtained from control and 1,2-dimethylhydrazine-treated animals.

Feeding studies involving cycasin have indicated that pretreatment with an antioxidant, n-butylated hydroxyanisole (BHA), provides protection against the carcinogenic effect of cycasin. The in vitro inhibition of colon alcohol dehydrogenase by BHA implies that this enzyme may be involved in the generation of the ultimate carcinogen from methylazxymethanol, a metabolite formed from cycasin and from 1,2-dimethylhydrazine. Initial experiments involved commercially available purified rabbit muscle L-glycerol-3-phosphate dehydrogenase and horse liver alcohol dehydrogenase and the antioxidants, n-butylated hydroxy-anisole, phenol and toluene, each at 0.05 to 1.0 mM final concentrations. L-Glycerol-3-phosphate dehydrogenase was inhibited 40% by 0.05 mM BHA and BHT while alcohol dehydrogenase was inhibited 40% by 0.5 mM BHP and BHT. This inhibition was observed under anaerobic conditions and after a 5-min preincubation of enzyme with inhibitor before the addition of NAD. Partially purified liver and colon cytosolic enzymes were prepared by affinity chromatography with Matrex Blue A and Red A. Both enzymes prepared from liver and colon were inhibited markedly by 0.5 mM BHA and BHP. Current experiments are investigating the relative activities of the two enzymes toward methylazoxymethanol. The results thus far indicate that more than one NAD-dependent dehydrogenase is inhibited by the antioxidants and that this inhibition is not tissue specific.

Regulation of intestinal energy metabolism -- The shuttle mechanisms for the transport of reducing equivalents from cytosolic NAD into the mitochondria have a major role in the generation of metabolic energy. Our results suggest a significant role for the L-glycerol-3-phosphate shuttle in the colon, but not in the liver. The rates of reducing equivalent transport by a reconstructed L-glycerol-3-phosphate shuttle were 11.2 and 6.9 nmol NADH/min-mg mitochondrial protein with mitochondria isolated from liver and colon, respectively. In contrast, a reconstructed malate/aspartate shuttle transported the equivalent of 34.8 and 0.7 nmol NADH/min-mg mitochondrial protein with liver and colon mitochondria, respectively.

In addition, the K_m of liver NAD-linked L-glycerol-3-phosphate dehydrogenase for dihydroxyacetone phosphate was determined to be 0.15 mM, which is much greater than the reported endogenous dihydroxyacetone phosphate concentration of 0.035 mM. The K_m of colon NAD-linked L-glycerol-3-phosphate dehydrogenase for dihydroxyphosphate is less than the reported dihydroxyacetone phosphate concentration.

Current studies examine the role of the two shuttles in isolated colon cells by monitoring the levels of pyruvate, lactate and other glycolytic intermediates in presence of inhibitors. The inhibitors aminooxyacetate and indomethacin are specific for the mitochondrial components of the malate/aspartate (GOT) and L-glycerol-3-phosphate shuttles, respectively. Alteration in metabolite levels by one or both inhibitors is an indication of major shuttle involvement in colon energy metabolism and will clarify the relative contribution of these shuttles to colonic energy metabolism.

Further study of NAD-linked L-glycerol-3-phosphate dehydrogenase, the cytosolic component of the L-glycerol-3-phosphate shuttle, was performed after partial purification by affinity chromatography using agarose immobilized Procion Red HE3B. Disc polyacrylamide gel electrophoresis revealed three distinct bands for liver and colon, only one of which was unique to each. Gel isoelectric focusing with broad range ampholytes showed bands centering near pH 3.0 for liver and pH 4.0 for colon. These tissue-specific differences are being examined further by heat inactivation gel filtration and other electrophoretic systems.

Absorption of lipids: The role of intestinal protein synthesis in the absorptive process is being investigated in two ways. First, in vitro protein synthesis is being monitored in isolated tip and crypt cells as a function of molecular weight and of subcellular origin. These experiments will distinguish a major factor in relative rates of protein synthesis, e.g., growing vs. non-growing cell, by use of double labelled experiments and the determination of ratios on electrophoretic profiles.

A preliminary study (by E. E. McConnell) indicated that lipid assimilation was altered by pretreatment with TCDD. We have studied the effect of TCDD (80 µg/kg gavage) on the synthesis of intestinal cell proteins in adult male Fischer rats. Seven days after treatment, half the control and half the TCDD-treated rats were given 1.0 ml of corn oil by gavage and sacrificed 1 h later. Duodenal cells were isolated and incorporation of ^{3}H-leucine into total cellular proteins was monitored in vitro. All the fat-fed animals were shown to have epithelial cells that contained numerous lipid droplets. In the control fat-fed animals, the ^{3}H-leucine incorporation into cellular proteins was twofold greater than that of the control animals (non-fat-fed). In replicate experiments, ^{3}H-leucine incorporation from a) TCDD-treated rats was inhibited by 17.7 and 4.4% and b) TCDD-treated, fat-fed rats was inhibited by 64.5 and 53.6%. Since lipoprotein biosynthesis is necessary for chylomicron formation and transit from the intestinal epithelial cell, the marked inhibition of protein synthesis in the TCDD-treated, fat-fed rats clarifies the time-course accumulation of lipid in the intestinal epithelial cell, and may be, in part, an explanation for the mechanism of starvation associated with TCDD treatment. Additional studies will examine the nature and site of the inhibition, possible changes in cell kinetics and alteration to other aspects of lipid metabolism that involve the intestines, liver and adipose tissue.

Collaborative studies -- The trichothecene T-2 toxin (3α-hydroxy-4β,15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichotec-9-ene) is the major component extracted from strain 921 of F. sporotrichioides which is responsible for the development of the symptoms of alimentary toxic aleukia in laboratory animals. Investigations were initiated to examine the in vitro effects of T-2 toxin and its hydrophilic

analogue T-2 tetraol on the respiration of isolated rat liver mitochondria (RLM). T-2 toxin (2.5 mM) and T-2 tetraol (10 mM) inhibited state 3 respiration (ADP present) and prevented DNP stimulation with each of four mediators, succinate, pyruvate plus L-malate, L-glycerol 3-phosphate or L-glutamate plus L-malate. If either toxin is added last, it inhibits ADP- or DNP-stimulated respiration. Preincubation of RLM with toxin for 4 min revealed decreased respiratory control ratios. T-2 tetraol (5 mM) had a greater effect or respiration after 5 min than the T-2 toxin (2.5 mM), and this effect was not mediator dependent. Thus, the inhibition of RLM respiration is not solely due to inhibition of thiol-enzymes. In contrast, T-2 toxin (5 mM) does not inhibit RLM respiration in the presence of ascorbate/TMPD. Also, the spectral changes monitored with and without T-2 toxin (0.5 mM) at 605-625($a+a_3$), 551-540($c+c_1$) and 430-410(b), indicate an additional similarity to Antimycin A with respect to site of action. Although low concentrations of T-2 toxin (0.05-0.5 mM) do not uncouple LM respiration, both T-2 toxin (2.5 mM) and T-2 tetraol (5 mM) markedly stimulate the mitochondrial, latent ATPase, but to a lesser degree than DNP (80 μM). The stimulation of the ATPase may result from a non-specific effect to the membranes. This is the only known toxic effect of these two trichothecenes that would explain the immediate cytotoxicity found with T-2 toxin exposure. The other known cytotoxic effect of the trichothecenes, including T-2, is the inhibition of protein synthesis.

In previous cross-fostering experiments, pregnant rats were exposed to gavage to 20 mg acrylamide/kg body wt.-day for 10 successive days (days 6 to 17 of gestation) and the marker enzyme lactate dehydrogenase (LDH) was measured in the intestinal tissue of control pups at 3 stages of development. The result was a statistically significant increase in LDH activity (treated dams -- control pups) which indicates a lactational effect. In order to investigate directly the passage of acrylamide to the offspring through lactation, a single oral dose (100 mg/kg body wt.) of [2,3-^{14}C] acrylamide (specific activity = 0.512 mCi/mmol) was administered to rats during the 14th day of lactation. Milk samples (100 μl) were taken by manual expression form control and treated rats at varying time periods from 15 min to 25 h after dosing. The spectrophotometric measurement of acrylamide in the protein-free milk extracts employed diazomethane and 4-dimethylaminocinnamaldehyde. Radioactivity and protein concentrations were also monitored directly in the milk of control and treated animals over this time-course. Protein levels gradually increased in the control and treated milk, 40 to 70 mg/ml. The results were similar time-courses for the radiolabelled material and chemical acrylamide with a peak at 45 min and a dramatic decrease thereafter. However, there was a slower decrease in the radiolabelled material than in the acrylamide which may be the result of increased levels of an acrylamide metabolite(s) in the milk.

Current studies examine the composition of acrylamide metabolites in the milk and also generated in vitro from intestinal and hepatic homogenates. The chemical analyses will be by HPLC.

The presence of esterase activity in the skin and intestines has stimulated interest in the toxicity of the phthalate acid esters and the monoester analogues. The monoesters of the phthalate esters appear to be potent inhibitors (0.1 mM) of the succinate-mediated oxidative pathway in isolated rat liver mitochondria.

PROPOSED COURSE: We plan to concentrate our efforts in fiscal year 1982 on follow-up studies based on some of our current projects. In particular, we are interested in studying (1) the regulation of protein synthesis in isolated intestinal cells as it relates to differentiation and absorption; and (2) the effects of TCDD on intestinal protein and lipoprotein synthesis and lipid metabolism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A better understanding of the basic principles of normal intestinal functions should permit greater appreciation for the unique roles of this organ is absorption and metabolism. This better understanding of normal function should lead to better methods for the detection of dysfunction and malabsorption. The possible interactions of ingested substances, both natural and metabolized forms are rapidly on the rise as the nature and degree of intentional and unintentional contamination of ingested substances increase. Currently, our laboratory is employing a variety of techniques to investigate the mechanisms of normal and altered intestinal function at the cellular, subcellular and molecular level.

Many of our results have long-range significance in providing a better understanding of normal intestinal absorption, morphology and metabolism. Other observations include results which may have toxicological and clinical utility for the medical community. Examples of the latter include our studies on the responses to oral exposure to 1,2-dimethylhydrazine, 2,3,7,8-tetrachlorodibenzo-p-dioxin and acrylamide as well as our in vitro studies with T-2 toxin and phthalate acid esters.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70200 -07 LP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cellular Mechanisms of Metal Toxicity

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.6	4.6	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

When animals are exposed to different trace metals for prolonged periods of time, each metal produces a biological response profile which specifically characterizes exposure to that metal. The objective of these studies is to assess and characterize response profiles based on a thorough understanding of subcellular mechanisms of metal toxicity and specifically to (1) define and correlate ultrastructural and biochemical responses in vivo which characterize exposure to toxic trace elements and (2) develop early, specific, and sensitive biochemical testing procedures that may be used to evaluate human populations exposed to environmentally important trace elements. Specific metals and areas of interest include the biochemical effects of cadmium and lead on mitochondrial and lysosomal structure and function. Studies have shown that renal lysosomes play a central role in mediating the uptake and toxicity of circulating cadmium-thionein to renal proximal tubule cells and in the development of the attendant low molecular weight proteinuria. Cytosolic lead-binding components were found in target tissues for this element and were partially characterized by gel chromatography and electrophoresis. The binding components appear to be a major initial intracellular compartment for lead entering the cells of target organs.

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy; X-ray microanalysis; biochemical assays of mitochondrial respiration; and lysosomal, microsomal, cytosolic, and mitochondrial enzymes. Sephadex and DEAE chromatography, electrophoresis, isolectric focusing and amino acid analysis of metal binding proteins from mammals and marine shellfish. Tissue analysis for metals by atomic absorption spectroscopy and plasma emission spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: The pathophysiology of cadmium-thionein nephrotoxicity -- The mechanism of cadmium-thionein (Cd-Th) nephrotoxicity was studied in rats by intraperitoneal injection of 0.6 mg Cd as Cd-Th per kg of body weight. Ultrastructural evidence of proximal tubule cell damage and sloughing was observed over a 3-day period following Cd-Th injection. Early changes, characterized by a large number of small vesicles and an increase in small lysosomes in the apical portion of the cells, were coincident with changes in renal functions, which involved the reabsorption of protein and sodium and the regulation of urine volume and pH. Alterations in these parameters occurred as early as 8 hr after injection of Cd-Th, prior to obvious signs of proximal tubule cell death. Characterization of the urinary proteins by isoelectric focusing and SDS-gel electrophoresis indicated that the abnormal excretion of protein could be accounted for by an inhibition of normal tubular protein reabsorption. Ultrastructural morphometric and biochemical data have suggested that proteinuria develops as a result of a Cd-induced inhibition of secondary lysosome formation and, indirectly, by an inhibition of lysosomal protease activity. In addition, studies have indicated that the toxic effects and cell death occur as a result of a Cd ion inhibition of basic cell biochemical functions, in particular, RNA synthesis, due to the rapid release of the Cd ion withing the cell following degradation of the thionein molecules. These results suggest that Cd-Th exposure provides a good system by which a Cd-mediated low molecular weight proteinuria can be rapidly induced and studied.

Subcellular binding of ^{203}Pb in rat kidneys - Detection and partial characterization of cytosolic lead-binding components -- The subcellular compartmentation of a tracer dose of ^{203}Pb was studied in kidneys of control rats and rats given a single intraperitoneal injection of Pb acetate (15 mg Pb/rat) 6 days earlier. Intranuclear and cytoplasmic inclusion bodies were observed by electron microscopy in proximal tubule cells of Pb-treated rats. Kidneys of rats injected with ^{203}Pb acetate (30 µg Pb/rat) 24 hr prior to sacrifice were fractionated into crude and purified nuclear, inclusion body, mitochondrial and cytosolic components. ^{203}Pb activity was two times higher in kidney homogenates of controls compared with Pb-treated rats. This ratio was also observed in the mitochondrial and crude nuclear fractions whereas cytosolic ^{203}Pb activity from control rats was five times higher than Pb-treated rats. Purified nuclear and inclusion body fractions from Pb-pretreated rats contained 10 and 25 times higher levels of ^{203}Pb radioactivity, respectively, compared with controls. Gel chromatography of the cytosolic fraction from control rats 2 hr after injection of ^{203}Pb revealed the presence of two major lead-binding components with molecular weights of about 11,500 and 63,000 daltons. These components were not observed in the cytosolic fraction of Pb-pretreated rats. The 11,500 dalton peak did not incorporate ^{14}C-leucine, and concomitant administration of cyclohexamide with ^{203}Pb did not

inhibit incorporation of ^{203}Pb activity, suggesting prior formation of the component. These studies indicate that formation of Pb intranuclear inclusion bodies alters the intracellular compartmentation of Pb and that previously undescribed cytosolic Pb-binding components exist in kidneys of non-Pb-treated rats. These cytosolic binding components were also observed in the cytosol of brain, a second target organ for lead toxicity, but not in liver or lung, suggesting a target tissue-specific localization for these lead-binding macromolecules.

Intramitochondrial binding of lead and its relationship to membrane structural and functional alterations -- The intramitochondrial binding of ^{203}Pb was studied in isolated rat kidney mitochondria incubated with Pb acetate (10^{-5}-10^{-3} M Pb). These data were correlated with ultrastructural changes in mitochondrial physical integrity and membrane binding of the fluorescent probes 8-anilino-1-napthalene-sulfonic acid (ANS) and ethidium bromide (EtBr). Specific activities of the outer membrane marker enzyme, monoamine oxidase (MAO), and inner membrane marker enzyme, cytochrome oxidase (CO), were measured to assess changes in the function of proteins that are integral components of mitochondrial membranes.

Physical fractionation of washed mitochondria showed that about 75% of the total bound ^{203}Pb radioactivity was present in the intermembrane space (IMS), 19-20% in the outer membrane (OM), 1.5% in the inner membrane (IM) and 0.2% with the matrix (M) at all Pb dose levels except 10^{-3} M Pb where a threefold increase in ^{203}Pb binding to the OM and IM was observed with an associated decrease in the IMS. These findings were correlated with a progressive increase in physical distortion of both OM and IM as monitored by isotonic ammonium molybdate staining and electron microscopy and decreased KCl swelling/contraction behavior at 10^{-4} and 10^{-3} M Pb levels. Respiratory function supported by glutamate was found to be more sensitive to inhibition than that supported by succinate.

Hydrophobic binding of ANS and EtBr to mitochondrial membranes under non-charged conditions was not markedly affected by Pb. The preferential inhibition of glutamate respiration by Pb was correlated with a 60% inhibition in increased EtBr fluorescence during state 4 glutamate respiration at 10^{-5} M and 10^{-4} M Pb but an abolition of increased fluorescence was observed for both substrate types at 10^{-3} M Pb. The membrane binding of ANS upon succinate addition followed a pattern similar to that for EtBr. MAO activity was increased to 700% of control at 10^{-3} M Pb, whereas the IM marker enzyme CO was decreased at only the 10^{-3} M Pb level to 16% of control. These data indicate that intramitochondrial Pb binding is influenced by variations in overall Pb concentrations with a concomitant alteration in the effect of Pb on the metabolic functions localized in specific membrane compartments. Lead binding to mitochondrial membranes appears to inhibit mitochondrial membrane metabolic activity by altering the energization capacity of the membrane and not via direct structural alteration.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal toxicity following prolonged exposure by combined ultrastructural and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations. In particular, metal-specific porphyrinurias

and proteinurias should indicate the early development of metal toxicity. Such indicators would have potential applicability to populations living near fossil fuel power plants.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 71000-02 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Estrogen Action in Liver

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

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SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	2.0	3.2

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 by characterizing cytosolic and nuclear estrogen-binding-proteins and correlating the presence of receptors with estrogen-mediated induction or repression of protein synthesis. Some functional biochemical components of estrogen 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 estrogen binding proteins and the mechanisms involved are investigated in whole animal and culture systems. Environmental estrogens such as zearalenol mycotoxins, DES, and methoxy-chlor are assessed for estrogenic potency in liver.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES AND METHODS EMPLOYED: I. Investigate the nature of cytoplasmic estrogen binding proteins in rat liver.

A. Specific estrogen receptors. Separation of specific receptors from other non-specific estrogen binding proteins is accomplished by ammonium sulfate precipitation. Specific binding characteristics 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 estrogen binding specificity	In vitro competitive binding

B. Investigate the nature of other non-specific estrogen binding proteins (EBP). Unlike classical estrogen target tissues, the liver contains high levels of high capacity estrogen binding proteins. Binding characteristics of EBP 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 receptors or EBP undergo sex differentiation. Studies outlined in Parts I and II suggest that levels of EBP 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 EBP to changes in hormonal milieu during a critical period of early development.

III. Study the nature and sex differentiation of nuclear estrogen binding proteins in rat liver. These studies investigate: 1) nuclear translocation of specific estrogen-receptor complexes into male and female rat liver nuclei and 2) differences in the nuclear translocation process between liver and classical estrogen target tissues. Nuclear binding is investigated in vivo and in vitro (tissue minces, cell free systems, isolated hepatocytes and isolated perfused liver). Binding is analyzed by density gradient centrifugation and exchange assays.

IV. Elucidate the role of EBP in the nuclear translocation process. These studies compare the rate of uptake and nuclear retention of estradiol receptor complexes in relation to the quality and quantity of EBP. Specific EBP profiles are produced by partial purification and endocrine manipulations of the neonatal adult animal. Techniques are the same as outlined in Part III.

V. Characterize the role that estrogen receptors and EBP play in biological, biochemical and physiological response of the liver to estrogens (including estrogenically active xenobiotics). Indicators of hepatic responsiveness are G-6-P-dehydrogenase, serum triglycerides, production of specific lipoproteins, production of renin substrate, production of α-2μ-globulin, RNA polymerase and biliary function. These parameters are studied in vivo in isolated hepatocytes and in isolated perfused liver. Additionally, estrogen-responsive forms of cytochrome P-450 will be investigated using in vitro translation systems.

VI. Elucidate the role of estrogen action in hepatotoxicity. The approach is to study the sequence of biochemical events which lead to a hepatotoxic response or toxicity in another organ system as a consequence of hepatic estrogen action. End points could be liver histopathology or dysfunction, cardiovascular disease or hypertension.

MAJOR FINDINGS AND PROPOSED COURSE: Adverse side effects of estrogenic compounds on the liver are receiving increasing attention. Certain of these estrogen-induced changes could reflect a direct liver-hormone interaction dependent upon the presence of estrogen receptors in the liver cells. Our studies demonstrate that liver cytosol fractions from both male and female rats contain estrogen-binding components possessing criteria assigned to receptor proteins. These criteria include a finite binding capacity together with a high affinity and binding specificity for estrogens. Ontogeny studies in rats have demonstrated that hepatic cytosolic receptor levels are low in the prepubertal period but increase rapidly at the time of puberty until adult levels are reached in 7-week-old animals. This increase in receptor levels is apparently not accompanied by a change in the physical and functional properties of receptor. Interestingly, male liver contains an additional estrogen-binding protein(s) distinct from the receptor. The ontogeny of this binding protein(s) is similar to that of receptor in males and the physiological significance of sex-specific binding proteins is under investigation. These binding proteins appear to be under pituitary-hypothalamic control.

Utilizing in vivo and cell-free systems, we have demonstrated that both male and female estrogen-receptor complexes undergo translocation into liver nuclei. In contrast to uterus, two conformational forms of estrogen-receptor complexes are evident in both male and female liver nuclei. The presence of receptors indicates that the liver is a target organ for estrogens. The study of hepatic estrogen-receptor interactions and the consequences of this estrogen action is clearly of importance in determining the impact of estrogenically-active chemicals on liver function. To further investigate the sex differentiation of hepatic estrogen-binding protein(s), (^{3}H) E_2-labeled cytosol from male or female rats was applied to Sephadex G-75 columns. The elution profile of labeled proteins shows a species (peak I, MW > 75 K) common to both sexes, which binds (^{3}H)E_2 at comparable levels to that of the specific estrogen receptor. In addition, three other species (peaks II, IV and V) are present in both sexes, with male levels being quantitatively greater than female. Also present is a species (peak III) found only in male cytosol. Competition studies demonstrate differences in steroid specificity for individual proteins. The elution profile of labeled cytosol from immature male liver resembled that of the adult female. Gonadectomy of adult male and female rats has no effect on the protein profiles. However, male rats which were

castrated one day after birth and sacrificed as adults exhibit a disappearance in peak II and marked reduction in the level of peak V. Hypophysectomy of adult rats results in the abolition of EBP sex differences. These studies demonstrate qualitative and quantitative differences in levels of 4S binding. The maintenance of these sex differences is pituitary-dependent and may be programmed at birth through the hypothalamic-pituitary axis by neonatal androgen exposure.

Our studies also have evaluated binding characteristics of three derivatives of the estrogenic mycotoxin zearalenone by components of liver cytosol. Cytosol from male and female rats contains similar amounts of specific estrogen receptors which sediment in the 8-9S region of 5-20% sucrose gradients. In addition, male liver cytosol contains a second class of higher capacity non-receptor estrogen-binding sites sedimenting in the 4-5S region of sucrose gradients. Sedimentation analyses show that each of the mycotoxin derivatives (P-1496, P-1502 and P-1560) behave in a manner analogous to the synthetic estrogen DES in that they compete effectively for 8S receptor sites while they bind poorly to 4S 'non-receptor' sites. Relatively, binding affinities for receptor sites were determined by competition studies. During a short (90 min) incubation period, the relative binding affinities of P-1496, P-1502 and P-1560 for receptor sites were 30%, 17% and 12%, respectively, of that exhibited by estradiol-17β. The relative binding affinity of estradiol-17β and P-1496 did not change as a function of time at 4°C. However, the relative binding affinities of P-1502 and P-1560 decreased to 6.8% and 4.2%, respectively, during an extended (18h) incubation period at 4°C. Each derivative exhibited similar relative binding affinities towards liver and uterine receptors during the extended incubation period. Dissociation rate constants were obtained indirectly by measuring the rate of exchange of the unlabeled ligand with [^{3}H]estradiol at 25°C. These studies show that the mycotoxin derivatives have the potential for modulating liver function through interaction with specific estrogen receptors. The estrogenic potential of the derivatives may depend upon the formation of a stable, slowly dissociating ligand-receptor complex.

Since the pituitary regulates various aspects of liver biochemistry, we have evaluated hypophyseal influences on levels of estrogen receptor (ER) and higher capacity lower affinity (HCLA) binding sites. Male or female rats were hypophysectomized (Hx) at 60 days of age and levels of cytosolic ER and HCLA binding sites determined at 90 days of age using ^{3}H-estradiol (E_2) and the dextran-coated charcoal method. Additionally, conformations of estrogen binding proteins were evaluated on 5-20% sucrose gradients. Results revealed that levels of hepatic E_2R were 9.0 ± 2.4 fmoles/mg cytosol protein in intact females compared to 3.4 ± 2.2 in Hx females. Likewise, levels of ER were 9.8 ± 1.5 fmoles/mg cytosol protein in intact males and 2.7 ± 1.8 in Hx males. Scatchard analyses of ^{3}H-E_2 binding to receptor revealed no effect of hypophysectomy on the K_d. Although no sex differences in hepatic ER were detected, pronounced postpubertal sex differences were evident in both total cytosolic binding of ^{3}H-E_2 and cytosolic HCLA estrogen binding sites which sediment in the 4-5S region of sucrose gradients; male levels were approximately 10-fold greater than females. Hypophysectomy abolished the sex differences in HCLA binding sites by increasing female levels and decreasing male levels. Treatment of Hx male or female rats with growth hormone (2 units/kg body wt, two times daily) reversed the effects of hypophysectomy on hepatic ER. For example, Hx female rats that had received growth hormone exhibited receptor

levels of 11.9 ± 3.2 fmoles/mg cytosol protein. This value is four times greater than Hx females and slightly higher than age-matched and sham-operated controls. In contrast, administration of prolactin, FSH, or LH to Hx rats had no effect on ER levels. Administration of growth hormone to Hx rats did not reverse the effects of hypophysectomy on HCLA binding sites. Our studies also demonstrate that ectopic pituitaries implanted under the kidney capsule of Hx rats also partially reverse the effects of hypophysectomy on ER. These studies demonstrate that growth hormone plays a key role in pituitary regulation of hepatic estrogen action.

Studies have also been undertaken to investigate E-responsiveness in both male and female rat liver. Intact male and ovariectomized (Ovx) female rats were treated with estradiol (E_2) by implanting sc. 8mm sections of silastic tubing containing either 10% E_2 (20-30 µg/kg body wt/day), 90% cholesterol or cholesterol alone. Following two weeks of E_2 exposure, the animals were killed and the response of the liver to E_2 determined by measuring (1) the levels of triglycerides and cholesterol in both plasma and the 3 major classes of lipoproteins, (2) RNA Polymerase A and B activity and (3) the distribution of E-receptors between the cytosolic and nuclear compartments by exchange assays. Levels of plasma triglycerides increased from approximately 50 mg/dl in control Ovx females to 120 mg/dl in E_2-treated animals. This increase in plasma triglyceride was confined to an increase in triglyceride associated with very low density lipoprotein (VLDL). In contrast, both plasma cholesterol levels and the activities of RNA Polymerase A and B were unaffected at this dose (10%) of E_2. Unlike the females, both triglyceride and cholesterol levels were increased in the male rat following E_2 exposure. A linear dose-response in triglyceride levels, from 50 mg/dl in control to greater than 150 mg/dl, was demonstrated with increasing doses (0-40%) of E_2. In contrast to triglyceride levels, a maximal increase in plasma cholesterol (HDL, LDL) was obtained by treating male rats with 10% E_2. Significant increases in nuclear occupancy by E-receptors were observed following E_2 administration. Previous studies have shown that following hypophysectomy (Hx) the levels of hepatic E-receptors decreases by 70%. Consistent with effects on E-receptors, circulating VLDL levels were unaffected by E_2 treatment of Hx, Ovx animals. In order to further understand the mechanism of pituitary regulation of liver E-responsiveness, we attempted to reconstitute E-responsiveness in Hx animals by administration of growth hormone or by transplantation of pituitaries under the kidney capsule. These results suggest that the E-mediated increase in VLDL may be a consequence of direct estrogen action on the liver.

Our studies also have utilized isolated hepatocytes and the isolated perfused liver as model systems to investigate estrogenic actions in <u>in vitro</u> systems.

In our liver perfusion studies, liver viability was monitored by measuring leakage of cytosolic enzymes (LDH, SGPT and total protein) into the perfusate. These experiments demonstrated that liver perfusion could be satisfactorily conducted for at least 90 min. Levels of ER, quantitated by the dextran-coated charcoal method, were similar in males (7.3 ± 1.4 fmol/mg cytosolic protein) and females (9.7 ± 2.8 fmol/mg cytosolic protein). In both sexes, liver ER levels decreased to approximately 35% of control values after a 90-min perfusion. Inclusion of insulin (5 µg/ml) provided partial protection against the loss of ER during perfusion. Translocation of ^{3}H-E_2 into nuclei was measured at various time

points and with various concentrations of ligand. These studies showed that, using a concentration of 4 nm E_2, nuclear translocation rates were approximately 2 fmol/mg DNA/30 min in both males and females. Prior hypophysectomy of rats (which decreased cytosolic ER) resulted in concomitant decreases in nuclear translocation rates of E_2R complex. Our studies demonstrate that the isolated perfused liver may be a useful model in the evaluation of hepatic action of endogenous estrogens as well as estrogenically active chemicals.

Nuclei of isolated hepatocytes also have been shown to take up and retain activated estradiolreceptor complex. These studies have demonstrated time-course and concentration-dependent aspects of the uptake process and have demonstrated that liver like the uterus that loosely bound (KCl extractable) binding sites are associated with weak or antiestrogens, whereas tightly bound (ethanol extractable) binding sites are associated with compounds that produce true estrogenic responses.

PROPOSED COURSE: Further studies will examine the biological/biochemical response of the liver following administration of 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 renin substrate, triglycerides, VLDL (very low density lipoproteins), glucose 6-phosphate dehydrogenase, α-2μ-globulin, and RNA polymerase. Estrogen action in relation to cell type and location will be studied. The quality and quantity of hepatic estrogen-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-binding proteins play in estrogen-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 toxic responses of the liver and other systems to endogenous estrogens and to determine if estrogenically active chemicals produce the same type of response.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During recent years it is becoming increasingly evident that the liver may be a toxicologically 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 biologic and/or toxic responses to estrogens in the liver is associated with specific forms of estrogen-binding proteins. 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.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80001-09 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Microsomal Mixed-Function Oxidase Systems: Specificity and Function

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO) from various sources. Present work involves the purification of cytochrome P-450, cytochrome b_5, NADPH-cytochrome P-450 reductase and NADH-cytochrome c reductase from rabbit pulmonary and hepatic microsomal fractions. Components of the MFO are being examined by UV-vis spectroscopy, electron paramagnetic resonance spectroscopy, SDS-gel electrophoresis, and by their activities in reconstituted systems. Structural and immunochemical properties of the enzymes are also being investigated. The long-range objectives of this work are to determine the influence of: 1) multiple forms of the enzymic components of the MFO systems, 2) endogenous compounds, and 3) exogenous compounds (substrates, inducers and inhibitors) on the substrate specificities of MFO systems from different tissues and species.

PROJECT DESCRIPTION

METHODS EMPLOYED: Cytochrome P-450 is purified from rabbit pulmonary and hepatic microsomes by procedures developed in this laboratory. The purification of other enzymes is accomplished by published methods which are modified as required.

MAJOR FINDINGS AND PROPOSED COURSE: 1. NADPH-cytochrome P-450 reductase from either rabbit liver or lung is the same enzyme -- NADPH cytochrome P-450 reductase, the flavoprotein that transfers electrons from NADPH to cytochrome P-450, was purified from rabbit hepatic and pulmonary microsomal preparations. The enzyme from either source has the same monomeric molecular weight (72,000 daltons), spectra, catalytic properties, flavin content, amino acid composition and C-terminal amino acids. Preparations of the enzyme from liver or lung form the same peptides when partially digested with proteases and have a chemically modified (blocked) N-terminal amino acid. The kinetics of the reduction of cytochrome c catalyzed by either preparation are identical with respect to cytochrome c, NADPH and inhibition by $NADP^+$ or the antibody to the enzyme purified from liver. A second form of NADPH-cytochrome P-450 reductase (molecular weight 68,000 daltons) can be isolated from either liver or lung. This reductase can be separated from the larger enzyme by chromatography on Sephacryl S-200. Under the conditions employed, the larger enzyme forms aggregates of greater than 210,000 daltons, whereas the smaller protein exists as a monomer. Several lines of evidence suggest that the smaller protein is derived from the larger, probably as a result of proteolysis. First, the peptides formed from each protein by partial proteolysis are, with few exceptions, the same. Second, the first three C-terminal amino acids of each protein are the same. Third, both proteins have the same immunochemical and catalytic properties except that the 68,000 form does not transfer electrons to cytochrome P-450. These results suggest that the N-terminal portion of NADPH-cytochrome P-450 reductase is required for the interaction of this enzyme with cytochrome P-450.

2. Rabbit pulmonary cytochromes $P\text{-}450_I$ and $P\text{-}450_{II}$ have distinctly different substrate specificities -- The substrate specificities of rabbit pulmonary cytochrome P-450 isozymes have been assessed by antibody inhibition in microsomal preparations and in purified systems. Metabolic activity has been measured for different substrates by one of three methods: product formation, complex formation with cytochrome P-450, or mutagenic activity in the Ames system. Several compounds examined are substrates only for cytochrome $P\text{-}450_I$. In purified systems, benzphetamine, 7-ethoxycoumarin and norbenzphetamine are metabolized only by $P\text{-}450_I$ and the metabolism of these substrates in pulmonary microsomal preparations is completely inhibited by the antibody to this isozyme. In contrast, 2-aminoanthracine, 2-aminofluorene and 2-acetylaminofluorene are metabolized to mutagenic products only by $P\text{-}450_{II}$ in both purified and microsomal systems. Both $P\text{-}450_I$ and $P\text{-}450_{II}$ catalyze the metabolism of 4-ipomeanol, aflatoxin B_1, p-xylene, N-hydroxyamphetamine and benzo(a)pyrene. The roles of $P\text{-}450_I$ and $P\text{-}450_{II}$ in the metabolism of 4-ipomeanol, a pulmonary-specific toxin, will be further examined, particulary with respect to the nonciliated bronchiolar epithelial cell, the target cell in vivo. The activation of aromatic amines by cytochrome $P\text{-}450_{II}$ will be studied in detail and the role of pulmonary acetyltransferase in the activation of 2-acetylaminofluorene will be determined.

3. The metabolism of p-nitroanisole is mediated by two distinct pathways of electron flow -- NADH-mediated O-demethylation of p-nitroanisole appears to proceed entirely via cytochrome b_5 and does not involve cytochrome P-450 reductase in rabbit hepatic or pulmonary microsomal preparations. The evidence for this is twofold: first, the increased rate of oxidation of cytochrome b_5 caused by p-nitroanisole accounts for the electron flow required for the metabolism of p-nitroanisole; second, the antibody to cytochrome P450 reductase has no effect on the NADH-mediated reaction. In addition, incubations containing purified NADH-cytochrome b_5 reductase, cytochrome b_5 and cytochrome P-450 catalyze the metabolism of p-nitroanisole when NADH is supplied as the cofactor. In microsomal preparations from rabbit liver, no evidence for the participation of cytochrome b_5 in the NADPH-mediated metabolism of p-nitroanisole has been obtained. In pulmonary microsomal preparations, however, inhibition of the NADPH-mediated reaction by the antibody to NADPH-cytochrome P-450 reductase requires that the reduction of cytochrome b_5 be inhibited to a rate that is less than the rate of oxidation. Such inhibition is only attained when the activity of NADPH-cytochrome P-450 reductase is inhibited by greater than 90%. Other differences between the roles of cytochrome b_5 in rabbit liver and lung are under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Through a detailed and complete characterization of the rabbit pulmonary cytochrome P-450-dependent monooxygenase system, major differences between the abilities of the liver and lung to metabolize and, in some cases, activate exogenous chemicals, are being elucidated. For example, it is now clear that the Clara cells of the lung contain a higher concentration of the enzymes required for the activation of 4-ipomeanol than do hepatocytes. This finding may explain the pulmonary specificity of this toxin. In addition, this research has provided both information (for example, substrate specificities of different isozymes) and preparations (antibody preparations and purified enzymes) that have been very useful to furthering the research efforts of other investigators at NIEHS.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80002-11 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems

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

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

It is the long-range purpose of this project to study how various chemicals and physiological changes affect xenobiotic metabolism by the body. This laboratory has concentrated its effort on the lung as a target organ for exposure to environmental stresses. Present studies include isolation of rabbit lung cell types for the purpose of studying localization of xenobiotic metabolism within the lung and toxication-detoxication mechanisms in individual cell populations. The following model enzyme systems are being used for study of individual xenobiotic metabolic pathways in lung cell populations: coumarin hydroxylase, 7-ethoxycoumarin deethylase, benzo(a)pyrene hydroxylase, epoxide hydrolase, and glutathione transferase. Different lung cell fractions (mixed cell populations) appear to have different metabolic profiles indicating possible differences in cytochrome content in the cell types. Mixed-function oxidase activity is now being studied and compared in cell fractions containing either 80% alveolar type II cells or 70% nonciliated bronchiolar epithelial cells (Clara cells).

PROJECT DESCRIPTION

METHODS EMPLOYED: Protease type I (Sigma) instilled into the trachea is used for dispersal of rabbit lung cells. Populations of cells are separated from the cell digest according to their rates of sedimentation by the technique of centrifugal elutriation. Other methods employed for cell separation include density gradients, phase separation, differential attachment to tissue culture plates, and affinity chromatography. Cells are studied using light microscopic (including fluorescence microscopy) and electron microscopic techniques. Spectrophotometric, fluorometric, and radiometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques have been developed to disperse and separate the many lung cell types in order to localize and study drug metabolism in individual cell populations. Present research has been directed toward obtaining relatively pure populations of alveolar type II cells and Clara cells since these cell types contain a majority of the pulmonary endoplasmic reticulum (where mixed-function oxidase activity seems to occur). Protease I (Sigma) is used to obtain a cell digest consisting of 20-30% alveolar type II cells and about 5% Clara cells. The cells are separated by size in an elutriator centrifuge yielding a fraction which is 50-70% type II cells (1-2% Clara cells) and another fraction which is 30% Clara cells (about 10% type II cells). The alveolar type II cells in elutriator fraction 2 have been further purified by Percoll density gradient centrifugation. The Clara cells in elutriator fraction 4 are separated further on a Percoll density gradient followed by a second elutriator centrifugation step. The alveolar type II cell fraction contains 7-ethoxycoumarin 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 (70% purity) and are being compared to what was found in the type II cells as well as in whole lung homogenate. Immunological techniques with cell fractions and antibodies to the purified cytochromes have been used to localize the rabbit pulmonary cytochromes $P\text{-}450_I$ and $P\text{-}450_{II}$ in the separate lung cell populations. With immunohistochemical methods and SDS-polyacrylamide gel electrophoresis, we have demonstrated that both cytochromes $P\text{-}450_I$ and $P\text{-}450_{II}$ are present in the isolated type II and Clara cells. Metabolism and covalent binding of 4-ipomeanol, a pulmonary toxin with specific in vivo Clara cell toxicity, has also been studied in the isolated cells. Metabolic activation and covalent binding were observed in both the isolated Clara and type II cells, although to a much greater extent in the Clara cells. Techniques to prevent loss of monooxygenase activity during cell isolation and culture are now being investigated. Future research will focus on the study of toxication and detoxication mechanisms within isolated cell populations and in single isolated cells in order to understand the ways in which specific chemical and physiological stresses alter these systems in 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.

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 changes caused by steroids, metal ions, or other chemicals, or physiological changes (age) on these cellular systems may aid our understanding of when the body is more susceptible to toxic agents and, on a cellular basis, how the body handles these insults. 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.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-08 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Xenobiotic-Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents

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

PI:	Roberta J. Pohl	Research Biologist	LP	NIEHS
	James R. Fouts	Research Pharmacologist	LP	NIEHS
	Marguerite Coomes	Staff Fellow	LP	NIEHS
Other:	Anja Norling	Visiting Fellow	LP	NIEHS
	Rebecca Weaver	Biological Technician	LP	NIEHS
	Michael Boyd	Head, Molecular Toxicology	LCPB	NCI
	Charles N. Statham	Cancer Expert	LCPB	NCI
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COOPERATING UNITS (if any)
Biometry Branch; Histology

LAB/BRANCH
Laboratory of Pharmacology

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.8	3.4	1.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The 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 and UDP-glucuronosyltransferase activities are measured in whole skin, epidermal cells or subcellular fractions of epidermal cells from hairless mice (Hrs/J). Epidermal cell types high in xenobiotic-metabolizing activity and/or cytochrome P-450 content are being identified. Changes in xenobiotic metabolism and/or in the content of mixed-function oxidase components after exposure (topical or systemic) of mice to various effectors such as ultraviolet radiation, polycyclic hydrocarbons, chlorinated hydrocarbons, and steroids are being investigated.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Epidermal cells were isolated by digestion with trypsin or pronase and separated into different populations by density gradient centrifugation. Homogenization prior to subcellular fractionation was aided by cell disruption in a French Press at 10000 psi.

MAJOR FINDINGS AND PROPOSED COURSE: Epidermal and sebaceous cells were freed from the skin of hairless mice by digestion (@ 37°) with trypsin, and separated by discontinuous gradient centrifugation into fractions enriched in certain cell types. From Percoll gradients, cells freed by 30-min tryptic digestion were separated into two fractions, one enriched in differentiated keratinocytes and one enriched from 10% to 30% in basal cells. From metrizamide gradients two fractions were obtained from cells freed after 60-min tryptic digestion, one containing approximately 80% sebaceous cells and one enriched to about 50% basal cells. 7-Ethoxycoumarin O-deethylase (7-EC) and benzo(a)pyrene hydroxylase (AHH) activities were relatively high in the sebaceous cell and differentiated keratinocyte fractions, and low in the basal cell fractions. This pattern of cell-specific monooxygenase activity was also seen in autoradiographs of mouse skin incubated with tritiated 4-ipomeanol, a substrate with a metabolite so reactive that it binds to protein at the site of its formation. 7-EC and AHH activities were markedly increased in all cell fractions 20 hr after the mice were treated topically with β-naphthoflavone (β-NF). Zymbal's glands, specialized sebaceous glands of rodent ear canal, were also found to have markedly increased 7-EC and and AHH activities after animals were treated with β-NF (rats, 2 days after 5 mg β-NF, IP). UDP-glucuronosyltransferase (4-methylumbelliferone substrate) and glutathione-S-transferase (1-chloro-2,4-dinitrobenzene substrate) activities were assayed in the fractions from metrizamide gradients. Conjugating activities were relatively higher (but less responsive to induction by β-NF treatment) in the sebaceous cell fraction than in the basal cell-enriched fraction.

Cells isolated from skin by pronase digestion were found to have a higher proportion of viable cells than did those isolated by trypsin (70-80% vs. 10-30% by fluorescein diacetate hydrolysis and ethidium bromide exclusion tests). Use of predominantly viable cells is desirable for at least two reasons: 1. Although the non-viable differentiated keratinocytes obtained from the Percoll gradient had high 7-EC activity, it is not possible to evaluate how this activity compares to activity in undamaged cells. 2. Viable cells maintain differences in density and are impermeable to gradient materials, characteristics which facilitate their separation into fractions based on differences in size and density. We will, therefore, use cells isolated by pronase digestion to refine our cell separation techniques in order to obtain more homogeneous cell fractions. Methods will also be developed for more specific identification of cell types. The conjugating enzymes and their cofactors in various cell fractions will be more throughly characterized.

Skin and epidermal cells from rabbit will be tested for the presence of cytochrome P-450 isozymes and cytochrome P-450 reductase by binding of specific antibodies.

Changes in cell-specific xenobiotic metabolism elicited by exposure of mice to environmental agents will also be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Persistent environmental pollutants, e.g., 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

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Fouts, J. R., Foureman, G. L., and Vom Scheidt, A. T.: Xenobiotic-metabolizing systems in aquatic species. Activities in extrahepatic tissue and effect of ellipticine on hepatic mixed-function oxidases in aquatic mammalian species. Bull. Mt. Desert Isl. Biol. Lab. 20: 68-71, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80005-08 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
In Vitro Metabolism of Xenobiotics by Selected Marine Species

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

PI:	John R. Bend	Chief, LP	LP	NIEHS
Other:	Louise M. Ball	Guest Worker	LP	NIEHS
	Gary L. Foureman	Biologist	LP	NIEHS
	Roberta J. Pohl	Research Biologist	LP	NIEHS
	Richard M. Philpot	Research Chemist	LP	NIEHS
	Cosette Serabjit-Singh	Research Chemist	LP	NIEHS
	James R. Fouts	SES	LP	NIEHS
	Phillip Albro	Research Chemist	LEC	NIEHS

COOPERATING UNITS (if any)
Biometry Branch; Laboratory of Environmental Chemistry; C. V. Whitney Marine Laboratory, University of Florida

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Comparative Pharmacology (Toxication-Detoxication Mechanisms)

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.7	0.9

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We are investigating the biotransformation of foreign organic compounds in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Both cytochrome P-450-dependent monooxygenase activities and alkene and arene oxide-metabolizing enzymes (epoxide hydrolase and glutathione transferases) are being characterized in control fish and in fish pre-exposed to polycyclic aromatic hydrocarbons. Some studies are being undertaken with purified cytochrome P-450 and with purified glutathione transferases from aquatic species. Emphasis is given to projects that will enhance our understanding of toxication-detoxication mechanisms.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1. To characterize the hepatic monooxygenase (MO) system and the glutathione transferases of representative species from Maine and Florida, before and after pretreatment with polycyclic hydrocarbon-type inducing agents.

2. To monitor individual teleost and elasmobranch fish for exposure to 3-methylcholanthrene (3-MC)-like inducing agents, by assaying hepatic benzo(a)pyrene hydroxylase activity in the presence and absence of the in vitro probe 7,8-benzoflavone, to attempt to correlate induction with possible exposure to environmental inducing agents.

3. To examine the MO components and membrane dynamics of little skate (Raja erinacea) hepatic microsomes in order to explain the greater thermolability, during in vitro assay, of hepatic MO from skate when compared to that from rabbit.

METHODS EMPLOYED: Differential centrifugation; enzyme purification; spectroscopic, fluorometric, and radiochemical assays; organ perfusion procedures; ion exchange; thin-layer, paper, high-pressure liquid chromatography; and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Work was continued to improve the isolation and partial purification of the various forms of cytochrome P-450 present in hepatic microsomes prepared from little skates (Raja erinacea). Following chromatography on columns of DEAE-cellulose, octyl-Sepharose and then DEAE-cellulose again, evidence was obtained for at least two, probably three, and perhaps even four, different forms of cytochrome P-450. However, even at this stage of purity (at best, 10 nmol/mg protein), the specific content of the cytochrome fractions was low, and the observed spectral and chromatographic differences may still be due to the presence of various impurities. SDS-polyacrylamide gel electrophoresis was used to estimate the monomeric molecular weights of the various major protein bands in these preparations; bands were regularly found at 51,000 ± 1000 daltons, 54,000 ± 1000 daltons and sometimes at 56,000 ± 1000 daltons.

2. We have adopted (and verified) the use of whole liver homogenate from flounder as a suitable enzyme source for identifying "induced" wild flounder. More than 50% of the fish assayed in 1978, 1979 and 1980 (June-August) had elevated 7-ethoxyresourufin O-deethylase (7-ERF) and benzo(a)pyrene hydroxylase (AHH) activities (the latter which was inhibited by in vitro 7,8-benzoflavone). We have shown that there is no correlation between sex, gonad wt/body wt ratio, size of the fish, spawning or time of the summer when fish were sampled and AHH activities in the flounder. Hepatic cytochrome P-450 levels are elevated only in those fish that are very highly induced, and there is also a hypsochromic shift in the wavelength of maximum absorption of the cytochrome P-450 difference spectrum in microsomes from these highly induced fish (i.e., cytochrome P-448). Uninduced wild fish can be induced by administration of polycyclic hydrocarbons and become biochemically indistinguishable from induced fish captured in the sea. There was no obvious correlation between hepatic monooxygenase activities and renal or intestinal monooxygenase activities in the same fish. (This may be due to sequestering of pollutants in the liver, with limited renal exposure). A positive

correlation (0.83) exists between hepatic AHH and 7-ERF activities in individual flounder. Using SDS-polyacrylamide gel electrophoresis, hepatic microsomes from winter flounder pretreated with β-naphthoflavone or 1,2,3,4-dibenzanthracene were shown to have a band of molecular weight (approximately) 57,000. This band was absent or barely detectable in those untreated flounder that had low hepatic AHH and 7-ERF activities whereas it was prominent in the "wild" flounder with elevated AHH and 7-ERF activities.

A substantial number (67/166) of untreated sheepshead assayed in Florida were also found to have elevated AHH activities which were inhibited in vitro by 7,8-benzoflavone, indicating possible exposure to a 3-MC-like inducing agent and suggesting that induction of the hepatic monooxygenase system of marine teleost fish is widespread, at least along the eastern coast of the USA, even in relatively pristine areas (e.g., Maine).

3. A glutathione transferase enzyme, which efficiently converts benzo(a)pyrene 4,5-oxide to its isomeric glutathione conjugates, was isolated from little skate liver and purified to homogeneity. Several other polycyclic arene oxides, including phenanthrene 9,10-oxide, pyrene 4,5-oxide, and benzanthracene 5,6-oxide, were also shown to be very good substrates for this enzyme. Although trans-7,8-dihydrobenzo(a)pyrene-7,8-diol-9,10-epoxide was also a substrate for the enzyme, turnover of this oxide was relatively slow compared to the K-region oxides; about one-hundredth the rate for benzo(a)pyrene 4,5-oxide. The stereoselectivity and the regioselectivity of the various glutathione transferases from little skate liver will be investigated using several K-region arene oxides and styrene oxide. This work is being done in collaboration with Dr. Oscar Hernandez of the Laboratory of Environmental Chemistry.

4. NADPH-cytochrome c reductase (previously shown to exhibit temperature sensitivity which correlates with the temperature sensitivity of d-benzphetamine demethylase activity in hepatic microsomes from little skate or rabbit) was purified from little skate hepatic microsomes and rabbit hepatic microsomes. The molecular weights, obtained by SDS-gel electrophoresis of the isolated proteins, of the NADPH-cytochrome c reductases purified from skate (74,000) and rabbit (72,000) liver differed, and the enzyme from the fish did not react with antibody to rabbit liver NADPH-cytochrome c reductase. Moreover, preliminary experiments suggest that the flavin of the skate reductase has an abnormal structure.

In future studies, the flavin structure of purified hepatic NADPH-cytochrome c reductase from the little skate will be compared, in detail, to that of rabbit hepatic reductase. Similar studies will be performed with stingray liver NADPH-cytochrome c reductase, which has the same molecular weight and major proteolytic fragment as the skate enzyme, but which has higher thermostability.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products, and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food web, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and

to their potential value and hazard as direct and indirect foodstuffs for man. Comparison of the enzymes involved in metabolizing xenobiotics in different species should lead to a better mechanistic understanding of the overall effects of xenobiotics on animals, and of animals on xenobiotics.

Moreover, a detailed understanding of metabolic activation and deactivation pathways in marine animals may allow us to predict those species most likely to be affected by carcinogens, mutagens, teratogens, or cytotoxins in the environment and allow us to use them as sentinel or early warning indicators of toxic environmental contaminants.

PUBLICATIONS

James, M. O. and Bend, J. R.: Polycyclic aromatic hydrocarbon induction of cytochrome P-450-dependent mixed-function oxidases in marine fish. Toxicol. Appl. Pharmacol. 54: 117-133, 1980.

Hernandez, O., Walker, M., Cox, R. H., Foureman, G. L., Smith, B. R., and Bend, J. R.: Regiospecificity and stereospecificity in the enzymatic conjugation of glutathione with (±)-benzo(a)pyrene 4,5-oxide. Biochem. Biophys. Res. Commun. 96: 1494-1502, 1980.

Bend, J. R.: Induction of drug-metabolizing enzymes by polycyclic aromatic hydrocarbons: Mechanism and some implications in environmental health research. In: Environmental Chemicals, Enzyme Function and Human Disease (Ciba Foundation Symposium 76, New Series). Amsterdam, Excerpta Medica, 1980, pp. 83-99.

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Yagen, B., Ben-Zvi, Z., Foureman, G., Hernandez, O., Ryan, A. J., Cox, R. H., and Bend, J. R.: The metabolism and excretion of glutathione conjugates of styrene oxide in the winter flounder, Pseudopleuronectes americanus, a marine teleost: Identification of the corresponding S-cysteine derivatives as major urinary metabolites. Drug. Metab. Disp. In press.

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Bend, J. R., James, M. O., Little, P. J., and Foureman, G.: In vitro and in vivo metabolism of benzo(a)pyrene by selected marine crustacean species. In Proceedings of the 11th International Symposium of the Princess Takamatsu Cancer Research Fund. In press.

Hernandez, O., Foureman, G. L., Cox, R. H., Walker, M., Smith, B. R., and Bend, J. R.: Stereo- and regioselectivity in the enzymatic conjugation of glutathione with (±)-benzo[a]pyrene 4,5-oxide. In Polynuclear Aromatic Hydrocarbons, Chemistry, Metabolism and Carcinogenesis. Vol. 6. New York, Raven Press, 1980. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80006-10 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Environmental Contaminants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites in Marine Species

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

PI:	Margaret O. James	Senior Staff Fellow	LP NIEHS
	John R. Bend	Chief, LP	LP NIEHS
Other:	John B. Pritchard	Research Physiologist	LP NIEHS
	Gary L. Foureman	Biologist	LP NIEHS

COOPERATING UNITS (if any)
Biometry Branch; C. V. Whitney Marine Laboratory, University of Florida

LAB/BRANCH
Laboratory of Pharmacology

SECTION
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 ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The long-range objective of this project is to study the in vivo uptake, distribution, metabolism, and excretion of single, purified radiolabeled environmental contaminants, such as 2,4,5-trichlorophenoxyacetic acid, polychlorinated biphenyl isomers, and hydrocarbons, in vertebrate and invertebrate marine species that serve as human food sources. The role of environmental temperature and exposure to other pollutants on the processes involved are also investigated. Particular attention is focused upon potential carcinogens, mutagens, teratogens, and cytotoxins that may occur as food residues due to exposure of aquatic animals to environmental pollutants.

* This project was terminated because Dr. James left NIEHS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80007-10 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals

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

PI:	John R. Bend	Chief, LP	LP	NIEHS
Other:	Brian R. Smith	Senior Staff Fellow	LP	NIEHS
	Craig Harris	"P" Appointment	LP	NIEHS
	Gary L. Foureman	Biologist	LP	NIEHS
	Richard M. Philpot	Research Chemist	LP	NIEHS
	Oscar Hernandez	Visiting Associate	LEC	NIEHS
	Richard Cox	Chemist	LEC	NIEHS
	Michael Boyd	Pharmacologist	LCPB	NCI
	Charles N. Statham	Cancer Expert	LCPB	NCI

COOPERATING UNITS (if any)
Biometry Branch; Laboratory of Environmental Chemistry; National Cancer Institute

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication/Detoxication Mechanisms

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 (200 words or less - underline keywords)

Several arene and alkene oxides are known to react covalently with macromolecules, including nucleic acids, and to transform cells in vitro, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the cytochrome P-450-dependent monooxygenases, which convert unsaturated hydrocarbons to epoxides, and the further metabolism of arene and alkene oxides by soluble fraction glutathione transferases and microsomal epoxide hydrolase in hepatic and extrahepatic tissues. The relative quantitative importance of these metabolic pathways is being studied at various levels of cellular organization (isolated cells, perfused organs, purified enzymes) in an attempt to understand the mechanisms of organ-specific and cell-specific toxicity mediated by compounds metabolized to epoxides.

PROJECT DESCRIPTION

OBJECTIVES: 1) To characterize in intact tissue the enzymatic pathways responsible for the metabolic conversion of polynuclear aromatic hydrocarbons to epoxides and for the decomposition of these reactive intermediates; 2) to assess the importance of these enzyme systems in protecting tissues from reactive intermediates; 3) to evaluate the influence of cellular integrity and intracellular location of enzymes on the metabolism of reactive intermediates; 4) to correlate dosage levels with activity of epoxide-metabolizing pathways and toxicity (including histopathology and covalent binding of radioactivity to macromolecules) and rates of excretion in isolated perfused organs; and 5) to study the regioselectivity and stereoselectivity of various glutathione transferases with selected alkene and arene oxide substrates.

METHODS EMPLOYED: Purified enzymes, subcellular fractions of homogenates from various tissues, isolated perfused livers, kidneys, and lungs, isolated cells and intact animals are used to study alkene and arene oxide formation, biotransformation and toxicity. Highly purified radiolabeled substrates are used in most experiments. Metabolite isolation, identification and quantitation are most frequently accomplished using high pressure liquid chromatography (HPLC) and scintillation counting. Detailed characterization (^{13}C-NMR, NMR, mass spectroscopy, etc.) and synthesis of metabolites, when required, are routinely accomplished in collaboration with the Laboratory of Environmental Chemistry (LEC). In studies investigating the relationships between biotransformation and toxicity total metabolic profiles are determined and covalent binding to RNA, DNA and protein is measured, but only as a crude index of the amount of reactive metabolite present/formed.

MAJOR FINDINGS AND PROPOSED COURSE: 1. [Ring-^{14}C(U)]-styrene oxide-glutathione conjugates were prepared and used to study subsequent metabolism and excretion of the conjugates in intact rats and in isolated perfused rat liver, lung and kidney preparations. With these techniques, the role of the liver and various extrahepatic tissues, including lung and kidney, in mercapturic acid synthesis and excretion has been evaluated.

2. A technique was developed for studying the ability of xenobiotics such as p-xylene to destroy pulmonary cytochrome P-450. One lobe of the isolated perfused lung is ligated and removed, prior to administration of the test substance, to determine the control level of the cytochrome. (Cytochrome contents of both lobes are equal and unaffected by the perfusion process.) This method overcomes the problem of large interanimal variations in pulmonary cytochrome P-450 levels. In this system, both p-xylene and p-tolualdehyde caused destruction of cytochrome P-450. However, it was demonstrated that o-xylene, ethylbenzene, mesitylene, 1,2,4-trimethylbenzene and 1,2,3-trimethylbenzene were all considerably more potent in the destruction of pulmonary cytochrome P-450 than was p-xylene.

Studies will continue on the relationships between metabolism of selected alkylbenzenes and the destruction of pulmonary cytochrome P-450 in the rabbit. Attention will focus on one of the trimethylbenzene derivatives which were found to be the most potent compounds tested in the perfused lung preparation.

3. The position-specific metabolism of p-xylene was studied in reconstituted monooxygenase systems containing cytochrome $P\text{-}450_I$ or cytochrome $P\text{-}450_{II}$ purified from rabbit lung. The major metabolite formed was p-methylbenzyl alcohol in each case although 2,5-dimethylphenol was also formed by each cytochrome. It seems possible that an epoxide-like intermediate may be involved in the destruction of cytochrome P-450 by the pulmonary monooxygenase system. Future experiments will use alkylated benzenes that are more potent in their ability to destroy cytochrome P-450, such as 1,2,4-trimethylbenzene.

4. We have begun to assess the stero- and regioselectivity of the rat hepatic glutathione transferases with radiolabeled d- and l-styrene oxide as substrates. Our initial results suggest that the l-enantiomer is the preferred substrate with purified rat transferases AA, B and C. The other transferases tested (A, D and E) showed no apparent discrimination between these two enantiomers although enzymes D and E turned the substrate over well. More detailed kinetic experiments will be performed with the various rat hepatic glutathione transferases and the two styrene oxide enantiomers. These experiments will also be extended to some polycyclic arene oxides, including benzo(a)pyrene 4,5-oxide.

In collaboration with Dr. Errol Zeiger of the Environmental Mutagen Testing Program, l-styrene oxide was shown to be considerably more mutagenic to Salmonella typhimurium, strain TA 100, than was d-styrene oxide (almost twofold more active in certain experiments). This indicates that steric preferences in the formation and subsequent metabolism of styrene oxide may have important toxicological implications. The stereochemistry of the cytochrome P-450-dependent oxidation of styrene to d- and l-styrene oxide will also be studied in collaboration with Dr. Hernandez of the Laboratory of Environmental Chemistry.

5. In collaboration with Drs. Michael Boyd and Charles Statham of the National Cancer Institute, we have shown that 4-ipomeanol, a lung edema-inducing factor occurring in moldy sweet potatoes, is rapidly transformed by perfused rabbit lungs to a reactive metabolite(s) that became covalently bound to tissue macromolecules (DNA, RNA, and protein) or was conjugated with GSH. Covalent binding to RNA and protein was extensive (1.5 and 2.6 nmol/mg dry wt, respectively) at a concentration of 55 μM ipomeanol in the perfusate. Covalent binding to RNA was dramatically decreased when a lower ipomeanol concentration (5.5 μM) was used. The covalent binding, which is believed to be the initial lesion leading to the pulmonary toxicity resulting from exposure to ipomeanol, was quantitatively similar in perfused lungs to that occurring in vivo. This finding supports the hypothesis that the pulmonary toxicity of ipomeanol results from properties of lung tissue per se and does not result from transport of reactive metabolites from other organs, such as the liver, to the lungs.

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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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80031-05 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Role of Altered Membrane Function in Xenobiotic Toxicity

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

PI:	John B. Pritchard	Research Physiologist	LP NIEHS
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	Bruce Fowler	Research Pharmacologist	LP NIEHS
	Soon-Ho Lee	Expert Research Biochemist	LP NIEHS
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	2.6	0.8

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Many aquatic animals are highly sensitive to specific xenobiotics. Such organisms are used as models to identify those physiological processes most sensitive to environmental pollutants. Since the exposed location and functional importance of cell membranes make them particularly susceptible to the toxic effects of foreign chemicals, we have focused primarily on the interactions of xenobiotics with membrane transport systems, particularly transport in epithelia where the coupled function of apical and basal-lateral membranes provides the basis of physiological control mechanisms. We have used kidney and gill since (1) function of these organs depends in large part on membrane transport, (2) they both play important roles in determining the rate at which many foreign compounds are excreted from the body, and (3) each is vital to the overall homeostasis of the organism.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1. To evaluate the hypothesis that alteration of membrane function may lead to disruption of physiological systems dependent upon such function.

2. 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 luminal membranes (BBM) by Ca^{++} precipitation and differential centrifugation. Basal-lateral 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 of gill tissue. These enzymes have now been solublized in Triton X-100 and will be reconstituted in proteoliposomes to examine both the basic mechanisms of transport and the interactions of the ATPase-associated ion fluxes with xenobiotics.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Renal transport: We have focused on two transport systems, the reabsorptive transport of sugars (a BBM function) and the secretory transport of sulfate (a BLM function). Our initial emphasis has been on characterization of the transport systems and comparison with mammalian systems. We have shown that reabsorptive transport of glucose and mannose is dependent upon the luminal Na^+ gradient for its energy supply. Transport of glucose is electrogenic and is driven by both the electrical and the chemical components of the Na^+ gradient. As determined by kinetic modeling and by direct flux measurement, the coupling ratio between Na^+ and glucose in both flounder and striped mullet is 2 Na^+ to 1 glucose. This value is in agreement with results recently presented for the chicken and the dog. However, there remains considerable doubt in other mammalian species whether the ratio is 1:1 or 2:1. Transport is stereospecific and, based on both cis-inhibition and trans-stimulation, there appear to be two distinct carriers. Each will transport glucose, but only one will accommodate α-methyl-glucoside. This system is comparable to the major glucose system in mammals. The second system is shared by glucose and mannose. Early evidence indicates that this system may also be found in mammals. Finally, we have been able to solublize the glucose carrier protein and incorporate it into proteoliposomes. This is a necessary first step in the isolation and reconstitution of this transport protein in defined lipid matrix. Thus, while there is still a good deal of work necessary to completely characterize the carrier system, we are in an excellent position to use reabsorption glucose transport as a model to

evaluate effects of foreign compounds on the luminal membrane of the renal tubule. Our initial focus for this work will be mercury, because of its well-defined chemical reactivity, DDT because of its high lipid solubility, and naphthalene, a representative polycyclic aromatic hydrocarbon.

Our second system, secretory sulfate transport by flounder kidney, is not yet as completely characterized, but results obtained thus far are very exciting. Sulfate is secreted in vivo with a T_{max} of 40 μmole/kg/hr, indicating that it is a carrier-mediated process. The plasma concentration giving half maximal transport is 0.7 mM, very similar to the K_m determined in vitro (0.9 mM). We have now begun to characterize the membrane events using BLM vesicles. These experiments demonstrate proton $SO_4^=$ cotransport (or $OH^-/SO_4^=$ antiport, which is an equivalent process) at the BLM, but no sodium dependence. This is the first instance of coupled transport at that face of the tubular cell. Such a coupled cotransport model is consistent with available evidence on events known to occur at other poles of the cell. It also explains the Na^+ dependence of $SO_4^=$ secretion in the intact tubule, whereas transport is not Na-dependent in the isolated membrane. In the intact cell, low internal H^+ concentrations are maintained by luminal Na^+/H^+ exchange. This in turn depends upon the Na,K-ATPase to maintain low cellular Na^+. Thus, both ouabain and Na^+-free medium should impair $SO_4^=$ secretion in the tubule, exactly as observed, but $SO_4^=$ uptake in the BLM vesicles should depend only on the H^+ (OH^-) gradient, not on Na^+. We are now beginning to look at the organic anion system to determine if it responds similarly to pH gradients in BLM vesicles. We will also use the $SO_4^=$ secretory system to focus on interactions of xenobiotics at the BLM in a similar fashion to that described above for glucose reabsorption.

2. Gill ion transport: Whereas the kidney of aquatic organisms plays the major role in excretion of foreign compounds and divalent ions, primary regulation of ionic and osmotic composition of the body fluids takes place via monovalent ion (Na^+, Cl^-) transport at the gills. The importance of these processes, plus the exposed location of the gill membranes, renders them particularly susceptible to effects of xenobiotics and suggests that gill ion transport may be a fruitful test system for membrane toxicity. We have previously shown that the Na,K-ATPase of blue crab gill is extremely sensitive to inhibition by organochlorine pesticides, particularly DDT which inhibits at concentrations as low as 0.1 μM. Altered osmotic regulation was seen after exposure to only 0.3 ppm in vivo.

Major emphasis in recent months has been on the second important transport system in the gill, that responsible for HCO_3^-/Cl^- transport. We have isolated a HCO_3^--dependent ATPase from blue crab gill. Its biochemical properties are similar to HCO_3^--ATPases from other transport epithelia. These properties include SCN inhibition (I_{50} = 4.8 mM), K_m for ATP (4.4 mM), K_a for HCO_3^- (8.9 mM), substrate specificity, and pH optimum (7.8). However, it offers unique potential for future work on the mechanism of anion transport and modification by foreign compounds, because it may be induced by transferring the crab to low salinity seawaters and it may be readily solubilized for incorporation into artificial membranes. The latter technique has proven very valuable for study of the relationship between Na,K-ATPase and cation transport. This will be the next step in our HCO_3^--ATPase work.

3. Other studies: In collaboration with Dr. Bruce Fowler, we have used our vesicle techniques to focus on changes in proximal tubule luminal membrane function following cadmium-metallothionein (Cd-M) exposure of rat. No evidence of decreased vesicle glucose transport was seen, even though p-aminohippurate uptake by the intact cell was depressed, suggesting that the initial effect of CD-M was within the cell and that the luminal membrane was not compromised.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of their functional importance and their exposure to the full extracellular concentration of potentially toxic chemicals, cell membranes are particularly susceptible to the effects of xenobiotics. Preparations such as the isolated flounder tubule and the BBM and BLM vesicles derived from those tubules offer unique opportunities to examine the development and mechanisms of such membrane toxicity. Furthermore, since many marine organisms are particularly sensitive to certain agents, e.g., the blue crab to organochlorines, these studies may also permit identification of systems particularly prone to disruption by environmental contaminants. Such studies may then (a) point to sites which might also be prone to damage in man and (b) serve as indicators or warning systems for the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80032-05 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Excretion and Toxicity of Xenobiotics to Marine and Terrestrial Species

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

PI:	John B. Pritchard	Research Physiologist	LP NIEHS
	J. Larry Renfro	IPA Research Physiologist	LP NIEHS

COOPERATING UNITS (if any)
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SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.7	0.8

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Marine and terrestrial vertebrates are used to examine the role of organic ion transport in the renal and hepatic excretion of environmental contaminants such as DDT, 2,4-dichlorophenoxyacetic acid (2,4-D), and the polycyclic aromatic hydrocarbon, benzo(a)pyrene (BP). Topics under investigation include examination of 1) the importance of renal and hepatic organic anion transport in the rate of elimination of xenobiotics or their metabolites; 2) the interference of foreign compounds with elimination of endogenous wastes or toxins; 3) the role of intracellular binding proteins, such as glutathione S-transferases, in transport and toxicity of organic ions; 4) the influence of metabolism on the route and rate of xenobiotic excretion; and 5) the impact of membrane transport-related cellular accumulation in the development of the xenobiotic toxicity in target organs. The role of transport in the elimination of xenobiotics from specific organs, e.g., brain, as well as from the whole organism, is also characterized.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

OBJECTIVES: 1. To evaluate the factors which determine the rate of xenobiotic excretion. These include active transport, metabolism, plasma binding, and intracellular binding.

2. To assess the consequences of the extensive accumulation of xenobiotics resulting from organic anion transport in the kidney and other organs possessing this transport system.

3. To determine the mechanisms leading to the toxicity of foreign compounds, with particular emphasis on the interactions of such compounds with membrane function.

METHODS EMPLOYED: Our primary approach is comparative. We utilize the unique attributes of lower vertebrates or invertebrates to develop simple model systems, such as the isolated flounder tubules, to examine the interaction of xenobiotics with physiological systems. We then test the general applicability of conclusions based on these models by applying them to mammalian test systems. In addition, we try to define each problem at several levels of organization from the intact animal and cell to isolated membrane vesicles. Thus, we are able to more completely evaluate the significance of effects observed at any one level.

We had previously isolated luminal membranes (BBM) from the flounder tubule for study of reabsorptive transport mechanisms. We have now developed techniques enabling us to isolate basal-lateral membranes (BLM) in good yield and purity from flounder kidney proximal tubules. Sequential use of differential centrifugation followed by discontinuous gradient centrifigation yields BLM enriched 10- to 15-fold in Na,K-ATPase, the BLM marker enzyme. This is a particularly important advance, since it allows us to focus on the BLM where secretion, particularly organic anion secretion, takes place. As previously shown, organic anion secretion is of critical importance in determining the rate of xenobiotic excretion.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Excretion of polycyclic aromatic hydrocarbons (PAH)--PAH are metabolized by marine teleosts and the P-450 mixed-function oxidase system (mfo) responsible for this metabolism may be induced. Analysis of position-specific metabolism has shown that the bulk (50-70%) of this metabolism in both control and induced fish takes place on the benzo-ring of benzo(a)pyrene (BP), producing forms closely related to the carcinogenic mammalian metabolites. Thus, the pattern of metabolites in both control and induced fish is very similar to that of mammals induced with PAH compounds.

Since the fish is capable of producing potentially carcinogenic metabolites from PAH, it becomes vital to assess the mechanisms controlling the excretion of these compounds. Using renal clearance techniques, we have shown that different early BP metabolites are excreted at very different rates from each other or from BP itself. For example, BP-7,8-dihydrodiol is excreted at a rate more than 10 times that for BP-7-phenol. Thus, clearly the pattern of metabolism of each PAH is critical in determining the extent of its retention and the rate of its elimination. These experiments also indicated that the metabolites actually appearing

in the urine are polar conjugates (predominantly glucuronides) and that the mechanism responsible for the rapid elimination of some metabolites, e.g., BP-7,8-dihydrodiol, is active tubular secretion via the organic anion transport system. This observation raised the possibility that other agents like the phenoxyacetic acid herbicides, which we have previously shown to inhibit organic acid transport, may also retard the elimination of BP and its carcinogenic metabolites. Initial tests of this hypothesis indicate that the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), is indeed an effective inhibitor. Thus, these results indicate that these herbicides, though often held to be relatively non-toxic in their own right, may potentiate the toxicity of other environmental chemicals by retarding their elimination from the body.

We are particularly anxious to extend these studies on PAH excretion. We will focus on (a) identification of all polar metabolites excreted and of the site(s) and determinants of their production. (b) We will also compare the renal handling of BP and its primary metabolites in control flounder with fish in which the P-450 mixed-function oxidase system has been induced. Using high pressure chromatographic analysis, we will be able to quantify the extent of metabolism, the retention of specific metabolites within the body, and the excretion of these compounds in urine and bile. In addition, since these are cold-blooded animals and metabolism is markedly slowed at lower ambient water temperatures, we will also be able to examine the effects of different rates of metabolism independent of induction of new enzyme. Thus, we should be able to assess the relative impact of metabolism on both the excretion of the compound and the retention of toxic metabolites. (c) The third major area of interest is an assessment of the interactions of anionic xenobiotics with PAH elimination. In particular, evaluation of the effectiveness of the widely used phenoxyacetic acid herbicides in reducing PAH excretion is very important.

2. Organ-specific toxicity secondary to active organic anion transport--We have previously shown that organic anion transport, in addition to facilitating elimination of many xenobiotics, often leads to extensive (20- to 50-fold) intracellular accumulation of the xenobiotic. Such accumulation would appear to increase the risk of toxicity at those sites possessing the organic anion system, notably kidney, liver, and choroid plexus. In collaboration with Dr. A. L. Krall, we have shown that several anionic xenobiotics, including 2,4-D, 2,4,5-T and the polar DDT metabolite, DDA, do indeed behave in this manner. Their structure is similar to 2,4-dinitrophenol (DNP) and like DNP they markedly alter mitochondrial function and tissue O_2 consumption in kidney, liver, and choroid plexus. At low concentrations (5-50 μM) they behave as protonophores, dissipating the H^+ gradients necessary to couple electron transport with oxidative phosphorylation. At higher doses (~100 μM) they also inhibit ADP-stimulated respiration. Associated with these changes are inhibition of mitochondrial Ca^{++} and Mg^{++} ATPase, altered Ca^{++} transport, and the sequential loading of two distinct binding sites. Most importantly, because of the extensive transport and resulting high tissue concentrations, plasma concentrations of only 0.1 to 5 μM (i.e., 0.03 to 1.4 ppm) are required to yield intracellular concentrations capable of altering mitochondrial function in kidney tubular epithelium, hepatocyte, or choroid plexus in vivo. Such results explain the simultaneous inhibition of both anion (competitive inhibition) and cation (decreased energy supply) transport by the choroid plexus reported previously.

Related studies in collaboration with Dr. L. O'Tuama indicate that, even in the neonate and the new-born, choroid plexus has already developed the capacity to transport organic anions. Both 2,4-D and DDA are accumulated very efficiently by the choroid plexus of these young animals (rabbits) and both inhibit choroid plexus 5-hydroxyindole acetic acid transport. Salicylates, too, interact here, but not acetaminophen or its metabolites. These results raise the possibility that choroid plexus may be involved, perhaps via uptake of anions, with subsequent respiratory inhibition, and altered CSF production in the development of encephalapathies such as Reyes' syndrome. Therefore, the interactions of anions, choroid plexus, and brain permeability will be further investigated using in vivo techniques such as ventriculo-cysternal perfusion as well as in vitro choroid plexus studies.

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 envrionment is vital in predicting the hazards of subsequent consumption of the species by man. Furthermore, the use of model preparations such as the isolated flounder renal tubule permits rapid assessment of the interaction of xenobiotics with renal function, or in the case of organic anions, such as 2,4-D, with other similar transport sites in the body. The confirmation of 2,4-D and DDA inhibition of choroid plexus transport of a normal, but toxic, brain metabolite in the rabbit is an excellent example of the predictive value of such a model system from the marine environment. Finally, the addition of the ability to study membrane transport in vesicle preparations will now allow us to study the details of the interactions of xenobiotics with membrane transport independent of the effects on other cellular organelles.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80037-02 LP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Drug and Xenobiotic Metabolism in the Lungs: Mechanisms and Modifying Factors

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

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COOPERATING UNITS (if any)

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SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this project is to study the effects of various chemicals on drug and xenobiotic metabolism in various types of cells in the lung. Of particular interest are those compounds which are metabolized by the cytochrome P-450 monooxygenases. The rat was chosen as the experimental animal because it, like man, is subject to induction of these metabolizing systems by certain drugs and chemicals. Factors which alter the metabolic activity of the lung may play an important role in certain environmentally caused diseases, including some types of cancer. Currently, different cell types, especially alveolar type II cells and nonciliated bronchiolar epithelial cells (Clara cells), are being isolated from rat lungs so that a detailed examination of the toxication-detoxication processes in purified cell types and in individual cells may be carried out. Different lung cell types have different intrinsic abilities to metabolize various chemical compounds, including benzo(a)pyrene, and these activities are inducible to different extents. Also, treatment of animals with monooxygenase inducers alters the balance of toxication-detoxication enzyme systems in the different lung cell types.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various protease solutions are used to digest lungs for preparation of individual cells. Separation of specific cell types is achieved by use of centrifugal elutriation, density gradient centrifugation, and polyethylene glycol-dextran phase separation techniques. Counting and sizing of cells is done with a Particle Data cell counter and channelizer. Enzyme assays are performed using spectrophotometric, fluorometric, and radioisotopic methods. Microspectrofluorometric techniques are being developed for single cell assays on a Zeiss fluorescence microscope equipped with a photomultiplier and monochrometers.

MAJOR FINDINGS AND PROPOSED COURSE: Fractions of rat lung cells enriched either in alveolar type II cells or nonciliated bronchiolar epithelial cells have been prepared. Type II cells have been purified to near homogeneity. Clara cells have been prepared approximately 50% pure. Both types of cells contain monooxygenase activity and metabolize benzo(a)pyrene and 7-ethoxycoumarin, although the intrinsic activity of Clara cells appears to be several times that of alveolar type II cells. Administration of β-naphthoflavone or 3-methylcholanthrene to rats 48 hr prior to sacrifice causes induction of aryl hydrocarbon hydroxylase and 7-ethoxycoumarin in whole lung homogenate and in both the Clara cell-enriched fraction and the alveolar type II cell fraction. Epoxide hydrolase activity has been found in the lung cell digest and purified Clara cells, but was below the level of detection in type II cells. Similarly, glutathione transferase activity was found to be at least twenty times higher in Clara cells than in type II cells. Although both epoxide hydrolase and glutathione transferase activities were not increased by pretreatment of animals with β-naphthoflavone, UDP-glucuronosyltransferase activity, which was present in both Clara cells and type II cells, was found to be increased following pretreatment of rats with β-naphthoflavone. Efforts are being made to further purify Clara cells and to develop methodology for measurement of xenobiotic metabolism in individual cells from rat lung using microspectrophotometric and microfluorometric techniques. Conventional radiometric and high pressure liquid chromatographic methods will be employed to determine if different populations of rat lung cells differ in their spectra of metabolites produced from benzo(a)pyrene and other compounds. The subsequent binding of activated metabolites to cell macromolecules will also be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung is a tissue which is in direct contact with the environment and a host of airborne xenobiotic substances, some of which are presumed to be harmful and to cause disease. How the lung (and the individual cells within the lung) handles such xenobiotics may determine whether or not these chemicals are ultimately harmful. This research is directed toward an understanding, in biochemical terms, of how the various toxication-detoxication processes occur in the lung and in the individual cells of the lung. In addition, an understanding of factors which modify or alter these processes will contribute to a rational basis for assessment of the risks to health posed by various xenobiotics in the environment.

PUBLICATIONS

Jones, K. G., Devereux, T. R., Smith, B. R., Fouts, J. R., and Bend, J. R.: Isolation of Alveolar Type II cells and Clara Cells from Rabbit and Rat Lungs. Characterization and Study of Xenobiotic Metabolizing Pathways. In Kroch-Dubois, G. (Ed.): Toxicology, in press.

Fouts, J. R.: Drug Biotransformation in Lungs and Isolated Lung Cells. Czechoslovak Journal of Occupational Medicine (Pracorni Lekarshi) 32: pp. 355-360, 1980.

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY
Summary Statement

The purpose of this laboratory is 1) to conduct studies on the structure and function of the normal respiratory tract, 2) to investigate mechanisms of injury and repair in respiratory tract tissues exposed to environmental toxins, 3) to study the pathogenesis of specific disorders of pulmonary tissues (e.g., pulmonary fibrosis). The ultimate goal is to obtain new insights into the pulmonary biology and pathobiology of the lung which will provide the scientific basis for designing rational measures for prevention of pulmonary diseases. To carry out this mission, the Laboratory of Pulmonary Function and Toxicology encompasses a diversity of scientific talents and interests including biochemistry, cell biology, toxicology, and pathology. Presently the LPFT consists of the following groups: Cell Biology, Endocrinology, Prostaglandin Metabolism, Biochemical Pathology, Structural Pathology, and Environmental Carcinogenesis.

BACKGROUND AND SCOPE

The respiratory tract is the organ system bearing the heaviest burden of environmental contamination brought about by urbanization and industrialization. It is the target for gaseous and particulate air pollutants originating from numerous sources. Substances entering the body by routes other than the airways (e.g., ingestion of food additives, parenteral administration of pharmaceuticals), may also cause severe toxic changes of respiratory tract tissues. Acute and chronic respiratory tract disorders are among the most common diseases with environmental etiology. Yet our understanding of the pathogenesis of even the major groups of chronic respiratory diseases: bronchitis, emphysema, pulmonary fibrosis, and bronchogenic carcinoma is still rudimentary. The respiratory tract is a complex organ system composed of multiple segments with a great number of cells with diverse functions, many of which are only vaguely understood. This complexity has made it difficult to investigate the respiratory and non-respiratory functions of the various lung tissues and cells, their unique metabolic characteristics, their life cycle and turnover, their susceptibility to injury, and their capacity for repair. It is clearly evident that new experimental approaches have to be developed to advance our knowledge in the field of pulmonary biology and pathobiology.

Studies in the pulmonary branch are conducted at various levels of biological organization. The major emphasis, however, is concentrated at the cellular and biochemical level. The research efforts encompass studies of normal cell and tissue functions as well as their perturbations by various environmental agents.

CELL BIOLOGY: The research efforts of this group are concerned with studies of cell differentiation and regulation of cell function of epithelial cells lining the conducting airways. Studies are carried out in vivo as well as in cell culture systems. One of the cell types currently under investigation is the Clara cell. Together with Dr. Plopper (Davis, California), it was shown that in rabbits, this cell type, which was believed to be largely confined to the peripheral bronchioles, occurs in all airways. In the rabbit it seems to be the major secretory cell of the tracheobronchial tree. The ultrastructural and cytochemical characteristics of Clara cells, as well as their distribution are presently being determined in 2 other species commonly used in the laboratory, namely, hamsters and rats. Biochemical and cell culture studies of isolated Clara cells are being planned.

Other investigations in pulmonary cell biology are concerned with regulation of cell replication and cell differentiation of airway epithelial cells in culture. Conditions have been established for long-term primary cultures of rat, hamster, and rabbit airway epithelium in defined media. The substrate dependence as well as the hormone and growth factor requirements of these cells have been determined. Of particular interest is the question, which cell type(s) is responsible for establishing the cultures. Therefore, the focus of these investigations has been on the early phase of culture establishment. During the first 48-72 hours, a marked loss in cell differentiation, internalization and loss of cilia, decrease or loss of mucus granules is observed, subsequently, DNA synthesis and rapid growth commences. Attempts are being made to determine which cells enter the proliferative cell pool and are responsible for the expansion and growth of the cultures.

Other studies are concerned with the biosynthetic activity of established cultures, particularly the secretory glycoproteins. Mucus-like glycoproteins secreted into the medium are being analyzed. It is hoped that the defined culture conditions with serum free medium will allow detailed investigation of the regulation of mucus biosynthesis and secretion.

The lungs of mammals are known to contain "neuro-endocrine" cells similar in histochemical and ultrastructural characteristics to those present in the intestinal tract. However, so far it has not been possible to assign either a definitive function or a secretory product to these cells. In the airways they occur either as single cells or organized into so-called neuroepithelial bodies (NRB). The difficulty in investigating these cells has been that they are a minority cell population dispersed among other epithelial cells of the conducting airways. Methods are being developed for isolation and separation of neuroepithelial bodies from late fetal rabbit lungs. Lungs are dissociated with collagenase, and cell fractions enriched in NEBs by unit gravity sedimentation procedures are obtained. Enrichment was demonstrated histochemically in cytocentrifuge preparations obtained from various fractions and by biochemical means i.e., measurement of the distribution of biogenic amines, their metabolites and APUD enzymes in the cell fractions.

ENDOCRINOLOGY: The research efforts of this group are also concerned with neuroendocrine cells of the lungs as well as with identification of peptide hormones that may be important for the control of various lung functions (e.g., mucus secretion, bronchial and vascular muscle tone). The previous finding by other investigators that NEB-cells respond to diethylnitrosamine (DEN) either directly or indirectly, as manifested by induction of NEB hyperplasia by repeated DEN injection was confirmed and extended. The studies suggest that NEBs increase in size and cellularity. Whether DEN exposure results in formation of new NEBs is not clear but seems doubtful. Attempts to culture the DEN stimulated neuroendocrine cells are underway.

The neuroendocrine cell population in the cricoid of the guinea pig was investigated. Its mucosal lining was found to contain 4-5% neuroendocrine cells which, based on ultrastructural appearance, seem to belong to one type. The cricoid might, therefore, be a particularly useful source of neuroendocrine cells for future studies.

A peptide with physalaemin-like immunoreactivity has been isolated from mammalian tissues. Using antisera to the amphibian tachykinin physalaemin, it was found that acid extracts from respiratory tract and intestinal tract tissues of several mammalian species (rabbit, guinea pig, rat) show reactivity as detected by radio-immune

assay. Similar reactivity was also found in the mucosa of canine, bovine and porcine tracheas. The immunoreactive material isolated from stomach and trachea was, however, shown to be chromatographically distinct from amphibian physalaemin. In immunohistochemical studies, antiphysalaemin antibodies were found to react with Brunner's glands, neuro-endocrine cells in the small and large intestines of guinea pigs and rats, and possibly with nerve fibers in guinea pig bronchi and the hypothalmus. Further chemical characterization studies of the putative neuropeptide are being initiated.

PROSTAGLANDIN RESEARCH: A major objective of the prostaglandin program is to study metabolism and transport of prostaglandins (PG) and related substances in the lungs. One of the key topics of investigation is to identify the factors controlling production of PG and thromboxanes (TX) by pulmonary endothelium which in turn relates to the control of intravascular thrombosis. It was found that in the isolated perfused rat lung doubling of the ventilatory rate results in a 3-4 fold increase of PGI_2 and TXA_2 release into the vascular perfusate. This is mostly due to an increase in PGI_2. Another important factor stimulating PGI_2 and TXA_2 release is the concentration of platelets in the perfusate. This was found to be related to the release of arachidonic acid (AA) from the platelets. An important new finding is that Vitamin K_3 specifically inhibits PGI_2 synthetase in porcine endothelial cells without, however, affecting formation of other PGs or hydroxy fatty acids (HFA). The effect of estradiol on production of PGI_2 by endothelial cells is presently being investigated.

Using a newly developed HPLC method for rapid separation of PGs the formation of AA metabolites was studied in a variety of species. Human lung was found to produce roughly equal amounts of PGI_2 and TXA_2 from either AA or PGH_2, rat lung was similar to human lung in this respect. In contrast guinea pig lung produced mainly TXA_2. Human lung was also found to produce large amounts of a yet unidentified HFA when incubated with AA. This was further enhanced by addition of Ca^{++} and ionophore to the incubation mixture. Studies to identify the HFA are currently underway.

Another major aspect of the Prostaglandin work is related to metabolism of xenobiotics including carcinogens by PG and TX synthetase, and to the possible role of PGs in tumor promotion. Co-oxidation of carcinogens by PG synthetase has been a subject of investigation for several years. Most recently it was found that the benzo(a)pyrene (BP) intermediate BP,7,8-diol is metabolized by PG-synthetase to diol epoxide I. This co-oxidation was markedly enhanced by addition of AA and was inhibited by indomethacin. It was shown, using the 10T 1/2 transformation system, that this increase in diol epoxide I formation correlated with a 10-fold increase in transformation indicating the biological significance of the co-oxidation reaction. Further studies suggest that diethylstilbestrol (DES), 2-aminofluorene, β-naphthylamine and other chemicals can also be substrates for PG synthetase. Studies are presently underway to determine the importance of AA dependent oxidation of xenobiotics for toxicity and carcinogenesis *in vivo*.

Various lines of evidence suggest that PG mediated reactions might be important links in the chain of events leading to tumor production. In this context, the effect of the promoting phorbol ester TPA on AA release and formation of PGs as well as HFA's is being studied in 10T 1/2 cells. Specific inhibitors of AA release and of PG formation are used to study their effects on promotion of BaP initiated cells.

BIOCHEMICAL PATHOLOGY: The research efforts in this program are mainly concerned with the origin and composition of the extracellular lining layer (ELL) of the lung. Recently, existing methods for the isolation of lamellar bodies from type II alveolar cells by discontinuous sucrose gradient centrifugation were refined to yield lamellar bodies with ultrastructurally intact lamellae, perilamellar membranes and amorphous cores. Studies using these preparations indicate that NADPH cytochrome C reductase may be an integral component of lamellar bodies and not, as was previously believed, a microsomal contaminant. The ability of intact lamellar bodies to synthesize phospholipids is currently under investigation.

Other studies are concerned with the fluidity of the membranous components of the ELL, using electron paramagentic resonance. It was shown that the membranous components of the ELL are highly fluid at body temperature. In contrast, dipalmitoyl phosphatidylcholine (DPCC) the major constituent exhibits low fluidity at physiologic temperatures. However, it was found that other phospholipids can markedly lower the fluidity of DPCC. Studies are continuing to identify the materials in the ELL responsible for the high fluidity of its membranous components.

A disease associated with severe abnormalities of the alveolar lining layer is pulmonary alveolar proteinosis. Studies of pulmonary lavage effluents from individuals afflicted by this disease showed multi-lammelated myelin-like structures (MS). These MS are composed of trilaminar membranes, separated by amorphous material. Using proteases and lipases, it was demonstrated that the amorphous material is composed mostly of proteins, while the membranes are composed of phospholipids. Since the structures are readily disrupted by various chaotropic agents, they seem to be held together by hydrophobic interactions rather than by covalent bonds. At present the data suggest that the MS from patients with alveolar proteinosis are closely related to tubular myelin. Comparison studies of the chemical composition of MS and normal constituents of the ELL are underway.

PULMONARY PATHOLOGY: This program is concerned with the pathogenesis of asbestosis and other pneumoconioses. Studies were initiated on the early translocation of chrysotile asbestos and silica, following inhalation exposure. Brief exposure for 1 hour resulted in fiber deposition on the surface of alveolar duct bifurcations. Within 1-5 hours after exposure, the number of free fibers or particles decreased rapidly. At the same time, the fibers and particles were translocated to alveolar epithelium, basement membrane and alveolar interstitium without any apparent involvement of macrophages. Subsequently, it was observed that fiber containing macrophages accumulated at alveolar duct bifurcations (~24 hrs), which is known to be the site of later pathologic tissue reactions to asbestos. This accumulation of macrophages was most conspicuous at the proximal alveolar duct bifurcations. Studies on macrophages obtained by lavage from silica exposed rats showed that the relative number of macrophages containing particles remains constant at about 60% for several weeks. The early translocation of asbestos fibers to the interstitium, the preferential accumulation of fiber containing macrophages at alveolar duct bifurcations and the long persistence of particle containing macrophages are all thought to be important elements of the pathogenesis of dust related lung diseases.

The cell membrane effects of chrysotile asbestos are being studied in an *in vitro* model. Sialoglycoproteins of red cell membranes are labeled with gold chloride

conjugated wheat germ agglutinin (WGA). It was found that within 1-4 hours of interaction of asbestos fibers with the labeled red blood cells, the number of WGA binding sites on the red blood cell membranes was drastically reduced. This and other findings support the view that the early phases of asbestos toxicity are mediated by sialoglycoproteins on the cell membrane. Similar studies are planned using epithelial cells and macrophages.

ENVIRONMENTAL CARCINOGENESIS STUDIES: This work is concerned with three major research topics: the induction and progression of malignant transformation in respiratory tract epithelium, tumor promotion, and the relationship of mutagenesis and carcinogenesis. The studies on epithelial cell transformation have been focused mostly on defining conditions required to effect neoplastic transformation in primary cell cultures of rat tracheal epithelium and to determine early phenotypic changes associated with transformation. Using defined media supplemented with growth factors and hormones and collagen for cell attachment, a direct alkylating agent, N-methyl-N-nitro-N-nitrosoguanidine (MNNG), was used as a transforming agent. With 2 or more MNNG exposures, several phenotypic changes could be regularly induced: 1) epithelial colonies with sustained proliferation; 2) marked increase in growth rate of exposed cultures; 3) "immortalization" of epithelial cell cultures. The substrate and nutrient requirements of the early transformed cells are being defined for the purpose of establishing selection procedures for transformants.

In collaboration with colleagues at the Oak Ridge National Laboratory, studies are being conducted, which might lead to antigenic markers useful for identification of neoplastically transformed tracheal epithelial cells. Mouse-rat hybridomas were established using spleen cells from rats immunized with a neoplastic tracheal epithelial cell line. Several hybridoma clones have been isolated which produce antibodies against the malignant cell line. These antibodies were found to cross react with several malignant tracheal cell lines but not with normal tracheal epithelium or several other malignant lines of different origin. Studies are underway to determine when during the transformation process the antigens appear.

The tumor promoter TPA is known to be strain and species specific (at least when used *in vivo*). While it is very effective in some strains of mice, TPA has little promoting effect in other strains or species including hamsters. *In vivo* and *in vitro* studies showed, however, that hamster epidermal cells are responsive to TPA (DNA synthesis, hyperplasia, inhibition of terminal differentiation) but became refractory to TPA stimulation upon repeated exposure. This is in contrast to the effect of TPA on skin from sensitive mice, in which hyperplasia is potentiated by repeated TPA application. These studies suggest the importance of sustained hyperplasia in the promoting effects of TPA. Other studies are concerned with the question whether tumor promoters cause genotypic effects. In collaboration with Dr. J. Parry, it was found that in the yeast Saccharomyces cerevisiae, TPA induced aneuploidy. Whether similar effects can be induced in mammalian cells remains to be determined.

The relationship between mutagenesis and carcinogenesis was explored in studies with 2-aminopurine and diethylstilbestrol (DES). 2-aminopurine, a potent bacterial and bacteriophage mutagen, had weak mutagenic effects (HPRT and Na^+/K^+ ATPase loci) in Syrian hamster embryo cells (SHE cells) and had little or no transforming activity. In contrast, 6-N-hydroxylaminopurine was a potent mutagenic and transforming agent in this system. On the other hand, DES while not mutagenic

in SHE cells, did cause significant neoplastic transformation. However, DES caused chromosome loss and aneuploidy which in turn may lead to neoplastic transformation. The relationsip between chromosomal abnormalities and neoplastic transformation will be studied further.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 25000-04 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cultured Epidermal Cells as a Model for Skin Carcinogenesis

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

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SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The majority of human neoplasms is thought to be due to environmental factors. There is overwhelming evidence that skin cancer, one of the most commonly occuring human cancers, is usually due to exogenous insult.

Tumor promoters, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA), are very active in enhancing epidermal carcinogenesis in mouse skin but not in other species. The role of promoting agents in human neoplasms is unknown. The purpose of this work is (1) to develop an in vitro system consisting of hamster epidermal cells to study mechanisms of neoplastic transformation by environmental carcinogens and tumor promoters, (2) to determine the basis for the species specificity of tumor promoters, and (3) to determine if tumor promoters can induce mitotic aneuploidy.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Tumor promotion studies of carcinogen sensitive Syrian hamsters (Telaco inbred strains 87.20 and 15.16) are performed by treating the back of the animals with initiating doses of benzo(a)pyrene or dimethylbenzanthracene followed by treatment two to five times a week with phorbol ester derivatives for up to one year. Histological and ultrastructural examination of hamster skin is performed at various times after treatment with phorbol esters. Similar studies are performed with F344 rats and DBA, C57Bl, CD, Balb/c and Swiss strains of mice.

Metabolism of ^{3}H-TPA is studied in hamster epidermis and in hamster epidermal cells in culture by treating the target cells with the compound for various time periods, extracting the TPA with organic solvents and then measuring the metabolites in the extract after thin layer chromatographic separation.

Epidermal cells are isolated from newborn Syrian hamsters (Lakeview or Telaco strain 87.20 or 15.16) by surgically removing the skin from animals, floating the skin (dermis side down) on 0.25% trypsin for 20 hours at 4°C, physically pulling the epidermis from the dermis and dissociating the epidermal cells by physical disruption. The epidermal cells are then filtered to a single cell suspension and plated in various media.

In order to measure the degree of differentiation, the number of cells with cornified envelopes is determined as a percentage of the total cell number. To do this, the cells are trypsinized and resuspended in buffer containing SDS and β-mercaptoethanol. This solution lyses all the cells except the ones with cornified envelopes which can then be counted.

For transformation studies, the cells are treated at different times with carcinogens such as benzo(a)pyrene, N-methyl-N-nitro-N-nitrosoguanidine, dimethyl benzanthracene or 3-methylcholanthrene and promoters such as phorbol esters.

MAJOR FINDINGS AND PROPOSED COURSE: TPA, which is a potent promoter of mouse epidermal carcinogenesis, is very weak or inactive as a promoter on hamster, rat, and guinea pig skin. In order to probe the basis for this species specificity, the effects of TPA on hamster epidermis and hamster epidermal cells in culture were studied and compared to the known effects of TPA on mouse skin. We first wanted to determine whether TPA was reaching the target cells or if it was metabolized. Our studies revealed that metabolic inactivation of TPA does not account for its lack of tumor promoting activity on hamster skin. In addition, hamster epidermis treated with 81 nmol of TPA responded in a manner analogous to TPA-treated mouse epidermis. A hyperplastic response of the hamster skin was observed reaching a maximum at 48 hours after TPA treatment. Ultrastructural changes similar to those induced in mouse skin by TPA were observed in hamster skin. Hamster epidermal cells grown in culture also responded to TPA. TPA inhibited terminal differentiation of hamster epidermal cells and stimulated DNA synthesis in the cultures. These results demonstrate that TPA is very active on hamster epidermis and epidermal cells in culture. Since tumor promotion requires long-term exposures of cells to TPA and all the above experiments were acute experiments, the effects on hamster epidermis of TPA treatment for 1 to 4 weeks were studied. The most significant difference between mouse and hamster skin is in their hyperplastic response to TPA. In the mouse there is a potentiation in

TPA-induced hyperplasia after two or more exposures to the promoter, while hamster skin adapts to the TPA treatment and no longer responds hyperplastically after multiple doses. The mechanism of this adaptation is unknown, but is currently under investigation. Examining differences in adapted and nonadapted hamster skin should be useful in understanding the mechanism of TPA promoting action. We believe that the role of sustained hyperplasia in tumor promotion is under estimated by several investigators.

Rat epidermis, which responds weakly to TPA-promoted epidermal carcinogenesis, also responds hyperplastically to a single exposure of 100 μg TPA, and to a lesser degree after multiple treatments, but does not show the potentiation observed in mice. In addition, other studies with different strains of mice have shown a relationship between responsiveness to TPA-promotion and TPA-induced skin irritation after repeated application of the promoter. We are presently investigating this relationship. Taken together these data appear to support a role for sustained hyperplasia in the promotion phase of epidermal carcinogenesis.

Whether tumor promoters can induce genotypic changes is unclear. TPA is not active in a number of mutational assays. TPA induces sister chromatid exchanges in mammalian cells but his has not been confirmed in some studies. It is assumed that sister chromatid exchanges are cytological indications of cellular recombinogenic events. Tumor promoter induce recombination might then play a role in tumor development by leading to homozygosis of recessive mutations in a cell with a heterozygous mutation. However, TPA is not recombinogenic in yeast. A genetic event other than gene mutations and recombination, which may be important in tumor promotion is a change in the numbers of chromosomes in a cell, i.e., aneuploidy. Such chromosome changes can lead to hemizygosity of recessive mutations or may possibly result in phenotypic changes due to an alterations in gene balance. The induction of mitotic aneuploidy can be readily detected in a genetic system employing the yeast Saccharomyces cerevisiae. In collaboration with J. Parry (Swansea) we have shown that TPA induces mitotic aneuploidy but not recombination. Furthermore, the ability of other phorbol ester derivatives as well as non-phorbol ester promoters to induce aneuploidy in this system correlates with their tumor promoting activity. Mezerein, which is very similar to TPA in inducing phenotypic cellular changes, but is only a weak tumor promoter is inactive in this aneuploidy assay. Our data support an hypothesis that tumor promoters act by stimulating the expression of recessive mutations (presumably in regulatory genes) produced by an initiating agent or by altering gene balance, both of which could result from changes in chromosome numbers as the result of chromosome aneuploidy. Such changes in chromosome numbers have frequently been observed in the karyotypes of tumor cells and our data suggests that such changes are causal in the process of tumor development. Such changes in chromosome aneuploidy would readily fit into multistage models of carcinogenesis.

The effects of tumor promoters on hamster skin and epidermal cells in culture will be continued. TPA induced stimulation of orthinine decarboxylase, arachidonic acid release and prostaglandin production, morphologically altered (dark) cells, and sustained hyperplasia will be studied and compared to results with mouse skin. To confirm that initiated hamster skin, which is not promoted by TPA, is indeed initiated by the carcinogen treatment 1) determiniation will be made of the extent of carcinogen-DNA adducts that are formed and 2) promotion by wounding will be attempted to confirm that this phenomenon can occur in hamsters.

Transformation and promotion of epidermal cells in vitro will be studied to provide a model system to study these processes in culture. Hamster cells are ideal for these studies due to a low rate of spontaneous transformation compared to murine cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since skin cancer is one of the most prevalent human neoplasms, one clearly influenced by environmental factors, finding a model to investigate the complex interrelationships between target cells, carcinogens, and promoting agents, as well as the progression from normal to preneoplastic to neoplastic cells would be of considerable value to the understanding of environmental carcinogenesis.

PUBLICATIONS

E. E. Sisskin and J. C. Barrett, Hyperplasia of Syrian Hamster Epidermis Induced by Single But Not Multiple Treatments with 12-O-Tetradecanoyl-Phorbol-13-Acetate (TPA). Cancer Research, 41, 346-350 (1981).

E. E. Sisskin and J. C. Barrett. Inhibition of Terminal Differentiation of Hamster Epidermal Cells in Culture by the Phorbol Ester 12-O-tetradecanoyl-Phorbol-13-Acetate. Cancer Research, 41, 593-603 (1981)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25001-04 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cellular and Molecular Mechanisms of Neoplastic Progression

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

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SECTION
Environmental Carcinogenesis

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

The objective of this study is to elucidate the cellular and molecular mechanisms of neoplastic development and to understand how environmental agents influence the progression of normal cells to malignancy. The specific aims of the research are: (1) identification of preneoplastic stages during neoplastic development; (2) determination of the role of somatic mutation in a multi-stage model of carcinogenesis; (3) elucidation of whether benign neoplastic cells progress to malignant cells; and (4) understanding the biochemical basis of cellular invasion.

The studies involve model systems of oncogenesis which employ fibroblasts and epithelial cells in culture.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: KD cells are normal diploid human fibroblasts and HuT-11 cells are a chemically induced neoplastic transformed derivation of KD cells. Both KD and HuT-11 cells were the generous gift of Dr. Takeo Kakunaga of the National Cancer Institute. Syrian hamster embryo cells were grown and assigned as described (PNAS. 75, 3297 1978).

Basement membrane (BM) from Syrian hamster lungs is purified by an extraction procedure employing acetic acid and N-lauryl sarcosine and used as a model substrate for invasion. Components of BM, Type IV collagen and glycoprotein, are isolated by either proteolytic digestion of intact BM for the former or by extracting with 8M urea for the latter. The extracted Type IV collagen is purified by gel filtration using 1M $CaCl_2$ in 0.05 M Tris HCl buffer of pH 7.5 on Biogel A-5m column followed by a CM-cellulose ion-exchange chromatography using a linear gradient of 0.02 M acetate and 0.1 M NaCl containing 1M urea. At each step of purification an SDS - polyacrylamide gel electrophoresis is done. An alternate procedures for the purification of Type IV collagen was developed by replacing 1M $CaCl_2$ with 2M Guanidine-HCl which gave a 95% yield of the protein.

The glycoproteins are isolated by extracting BM with 8M urea followed by gel filtration on Sephadex G-200. Purified glycoproteins are identified by SDS-agarose polyacrylamide gel electrophoresis using periodic acid-Schiff reagent and Coomassie blue stain for detection.

BM was radioactively labelled using ^3H-Na BH_4 by reductive alkylation yielding a specific activity of 1 x 10^6 cpm/mg protein and used as an invasion substrate. Assays of fibrinolytic activity and collagenase activity are performed by established procedures.

MAJOR FINDINGS AND PROPOSED COURSE: To test the hypothesis that genetic instability results following chemical transformation of human fibroblasts, we have determined and compared the mutation rates of a normal diploid human skin fibroblast (KD) and a chemical inducted transformed line (Hut-11) derived from KD cells. Both lines were generously supplied by Dr. Takeo Kakanuga, NCI. The two genetic loci used in this study were hypoxanthine phosphoribosyl-transferase (HPRT), an X-linked recessive locus, and Na^+/K^+ATPase, an autosomal dominant locus. HPRT mutants were selected by resistance to 6-thioguanine and Na^+/K^+ATPase mutants were selected by resistance to ouabain. Our growth conditions permit routine cloning efficiencies of 70-90% and population doubling times of 16-17 hours with both normal and neoplastic human cells. Mutation rates were determined by Luria-Delbruck fluctuation analysis. The HPRT mutation rates of KD($1.6\text{-}2.1 \times 10^{-6}$/cell/generation) and Hut-11 ($1.0\text{-}1.8 \times 10^{-6}$/cell/generation) cells were not different and compare favorably with previously published rates at this locus. The Na^+/K^+ATPase mutation rates of KD($3.8\text{-}8.5 \times 10^{-7}$/cell/generation) and Hut-11($6\text{-}13 \times 10^{-7}$/cell/generation) cells were also similar. The observed Na^+/K^+ATPase mutation rates are from 5-26 fold higher than previously reported. We feel that our improved growth conditions allow for the growth of previously undetectable ouabain resistant mutants. MNNG induced HPRT mutation frequencies and expression times were also similar. Increased mutation rates do not appear to be a necessary factor in carcinogen induced transformation of human cells.

Using Syrian hamster embryo fibroblast, which are amendable to concomitant studies of somatic mutation and neoplastic transformation, we have studied compounds which have been reported to be mutagenic but not carcinogenic or carcinogenic but not mutagenic. This approach should allow a critical determiniation of the relationship between mutagenesis and carcinogenesis without the complications that exist in comparing the two processes in vastly different assay systems. 2-Aminopurine (2-AP) is a classical mutagen in bacteriophage and bacteria, but is not carcinogenic in animals. We have shown that 2-AP induces somatic mutations and morphological transformation of mammalian cells in culture, but its activity is very weak for both endpoints. This may explain its lack of activity in animal carcinogenicity tests. A related compound, 6-N-hydroxylaminopurine (6-HAP) is a much more potent mutagen and in this system induces significant morphological transformation, which correlates with its carcinogenicity in animals. These results indicate that base analogs do have the potential to be carcinogenic, which strengthens the correlation of mutagenicity and carcinogenicity.

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 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 a point mutation, a frameshift mutation, or a small deletion. Of course, other mutagenic changes, which are not detected by these two mutational markers, may still be important. One example of this is a chromosome mutation. DES is known to induce genetic damage in cells by chromosome loss, and a recent report demonstrates a correlation between aneuploidy and vaginal cellular dysplasia in young women exposed prenatally to DES. The role of aneuploidy conversion in initiation of cell transformation is currently under investigation. Karyotypic alterations and cell cycle specificity of DES induced transformation are being examined. The ability of other agents to induce transformation, mutation, and aneuploidy is being studied as well.

Basement membranes, which from the amorphous extracellular matrix lining of all epithelial and endothelaial surfaces are a principal barrier to invasion of malignant neoplasms. We have examined whether malignant fibrosarcoma cells (BP6T) have the ability to degrade basement membranes. A convenient method involving perihepatic or perirenal implantation of cells was developed to demonstrate cellular invasion in vivo. BP6T cells were highly invasive by this assay and the cells are also highly invasive on the chick chorioallantoic membranes. Ultrastructural electron microscopic studies (done in collaboration with Dr. Brody, LPFT, NIEHS) clearly show disruption of basement membranes in the vicinity of invading BP6T cells.

To determine the mechanism of this process, an in vitro model was developed to study the degradation of basement membranes by tumor cells. Pulmonary basement membranes were isolated from hamster lungs by extraction with acetic acid and N-lauroyl sarcosine. The purified basement membrane was characterized according to its glycoprotein and collagen components. Radiolabeled basement membrane was

prepared by reductive alkylation with ^{3}H-$NaBH_4$. The specific activity of the ^{3}H-basement membrane was 10^6 cpm/mg protein with >80% of the radiolabel in the glycoproteins. BP6T cells, but not normal cells, were shown to degrade basement membranes extensively. This degradation was mediated by cellular plasminogen activator, an enzyme present in many malignant cells. We have shown that plasminogen activator does not play a role in growth control, as originally suggested by others, but the results with basement membrane degradation indicate a role for the secretion of plasminogen activator in the pathogenesis of invasive tumor cells.

Tracheal papillomas can be readily produced in hamsters by injection with diethylnitrosoamine. Preliminary studies indicate that these cells can be grown in vitro at least for short time periods. These studies will be extended to optimize growth conditions for papilloma cells. A comparison will be made of the growth properties of these cells with malignant tracheal carcinoma cells (induced in hamsters by N-nitroso-N-methylurea). It is the aim of these studies to develop a system for the growth of papilloma cells and the measurement and selection of malignant variants that arise from these cells. The biochemical assays for invasion (discussed above) will hopefully be used to detect these variants.

We are developing a quantitative system for the study of for progression of rat tracheal epithelial cells in culture. We have defined conditions which allow the growth of these cells with a fibroblast feeder layer. Under these conditions the normal tracheal cells grow with a high cloning efficiency (~20%) and growth rate. Carcinogen altered cells can be selected by their growth in the absence of the feeder layer support. Preliminary results indicate that this selection system can be used to quantitate carcinogen altered cells. These studies are being continued and the role of mutagenesis in this transformation process will be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fifty to ninety percent of all human cancers are environmentally related. Before effective programs in cancer prevention and control can be formulated, a better understanding is required of the mechanisms by which environmental agents induce and promote neoplasia. Since cancer is primarily a cellular disease, this project utilizes model systems of cells in culture to study the cellular and molecular mechanisms of environmental carcinogenesis. Of particular interest is the cellular development of neoplasia. The progressive nature of neoplastic transformation recently has been recognized but the role of environmental agents in this process is unclear. This problem is central to environmental carcinogenesis and is the focus of this project.

PUBLICATIONS

J. C. Barrett, A. Wong, J. A. McLachlan, Diethylstilbestrol Induces Neoplastic Transformation of Cells in Culture Without Measureable Somatic Mutation at Two Loci, Science, in press.

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J. C. Barrett. Cell Transformation, Mutation, and Cancer, in "The Use of Mammalian Cells for Detection of Environmental Carcinogens, Mechanisms and Application." ed. by C. Heidelberger, N. Inui, T. Kuroki and M. Yamada, Gann Monograph on Cancer Research, in press.

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J. C. Barrett, S. Sheela, and P. L. Carl. Possible Role of Plasminogen Activator and Other Proteases in Metastasis, in "New Developments in the Study of the Biology of Metastasis", ed. by I. A. Hart and L. A. Liotta.

T. Tsutsui, B. D. Crawford, P. O. P. Ts'o and J. C. Barrett. Comparison between Mutagenesis in Normal and Transformed Syrian Hamster Fibroblasts. Differences in the Temporal Order of HPRT Gene Replication. Mutation Research, 80, 357-371 (1981).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25002-04 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

In Vitro Carcinogenesis and Promotion Studies with Respiratory Tract Epithelium.

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

P.I.	V. E. Steele	Senior Staff Fellow	LPFT NIEHS
Others:	P. Nettesheim	Chief	LPFT NIEHS
	S. B. Pai	Visiting Fellow	LPFT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
N.I.H., N.I.E.H.S., Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of these studies is to define the various phases of respiratory tract carcinogenesis at the cellular and molecular level using in vitro systems. We have previously shown that the malignant transformation of rat tracheal epithelium could be studied in its entirety under well-controlled culture conditions. Subsequently we showed that we could enhance transformation using a well-known tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate thus demonstrating multistage carcinogenesis. This in vitro system has now been refined from organ-culture-exposed epithelium to monolayer-exposed epithelium thus allowing more quantitation and precision as to cell numbers at risk and cytotoxicity determinations. Early following carcinogen exposure we have found that there is a profound change in morphology and growth kinetics of these cells when compared to control. The elevation of growth and the percentage of cultures capable of continuous subculture was dose dependent. We also found that substrate requirements and nutritional requirements change markedly. These nutritional and hormonal requirements continue to change from the premalignant to malignant stages. We are currently utilizing these markers to investigate mechanisms of carcinogen dose response and tumor promotion in cultured airway epithelium.

PROJECT DESCRIPTION

METHODS EMPLOYED: Epithelial cells are dissociated from freshly-excised F344 rat tracheas and plated at 10^4 cells per 60 mm collagen-coated tissue culture dish. At various times after plating the monolayer cultures are exposed to n-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 1 or 3 hrs., rinsed twice and refluided with complete medium. Cell number determinations were either directly performed by dissociating the cultures and counting the cells in a homocytometer or indirectly using a grid eyepiece to determine cellular density and area. Cell lines were established by attempting to enzymatically dissociate the cells and subculture them at least five times. Tumorigenicity tests on these cell lines were performed by inoculating 10^6 cells into nude mice.

MAJOR FINDINGS AND PROPOSED COURSE: Using a monlayer culture of airway epithelial cells we have found that in vitro exposure to the direct acting carcinogen MNNG causes pronounced alterations in cellular morphology and growth. While the solvent exposed control cells become large differentiated squamous-like cells which slough into the medium, the carcinogen-exposed cells are small, have a high nuclear-to-cytoplasmic ratio, dense cytoplasm, and grow in densly packed sheets. The dramatic and sustained elevation in growth rate of exposed cultures could be observed as early as 2½ weeks following exposure. This change in growth control occured long before neoplastically transformed cells could be demonstrated and represents a very early stage of epithelial transformation. By 2½ weeks, following a single MNNG exposure on Day 1, the number of cells per dish was 10 fold higher than in control cultures. Multiple carcinogen exposures dramatically increased this cell number in a dose dependent manner. Between Days 30 and 90 we attempted to subculture these cells and establish cell lines. Control cultures produced no cell lines, while the percentage of cultures yielding cell lines increased form 17 to 70% as a function of the number of exposures. Tumorigenicity testing after 5 and 15 subcultures is not complete. We plan to continue these studies by examining the cellular kinetics following both single and multiple exposures to explain the mechanisms behind the dose effect.

Using the heterotopci tracheal transplant model we have found that cells exposed to DMBA for 4 weeks in vivo then placed in vitro without collagen substratum grow to form munerous large colonies, while control cells fail to survive for 30 days. These carcinogen altered cells also lost their requirement for 3T3-fibroblast conditioned medium, a requirement for control cells. The DMBA exposed cells could even grow immediately following exposure in a serum-free hormone supplemented medium, although growth is not optimal. These very early changes in substrate and nutritional requirements allow us to select out only those cells which ave been altered by the carcinogen for further study. We will use these cells to study changes in membrane structure and function as an early stage in the neoplastic process.

In a collaborative study with Dr. Wu, LPFT, we have determined a serum-free culture condition in which to study nutritional changes in tracheal epithelial cells as they progress from non-tumorigenic cell lines to fully transformed cell populations capable of forming malignant tumors in vivo. We have found that cells of idffering tumorigenic potential have different absolute requirements for the basic hormones and growth factors present in the medium. Also the response to added hormones is markedly different. These results indicate either changes in nutrient

uptake or utilization and may explain why tumor cells have a distinct growth advantage over normal cells in the in vivo situation. We hope to use other sets of cell lines to test the generality of the phenomenon and finally to study the molecular mechanisms responsible for these changes in tumorigenic behavior.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Respiratory tract carcinogenesis is probably the most important example of carcinogenesis in man since its epithelium is the critical site for cellular interaction with toxic environmental contaminants. Our current studies have focused on early indicators of neoplastic progression. These early markers point out discrete changes in carcinogen-altered cells which facilitate mechanistic studies of dose and neoplastic progression. Additionally these early changes might be useful as a diagnostic tool or a possible short-term carcinogen screening assay system with a relevant cell type.

PUBLICATIONS

Steele, Vernon E., Marchok, Ann C., and Nettesheim, P. Enhancement of Carcinogenesis in cultured Respiratory Tract Epithelium by 12-O-Tetradecanoylphorbol-13-acetate. Int. J. Cancer 26: 343-348, 1980.

Steele, Vernon E. and Nettesheim, P. Unstable Cellular Differentiation in Adenosquamous Cell Carcinoma. J. Natl. Cancer Inst. (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25004-04 LPFT

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Tracheal Epithelial Cells in Culture: Growth and Differentiation

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

PI:	Reen Wu	Research Chemist	LPFT	NIEHS
Others:	D. Smith	Technician	LPFT	NIEHS
	L. Y. Chang	P. Appt.	LPFT	NIEHS
	M. E. Porter	Biologist	LPFT	NIEHS
	J. Groelke	IPA	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Pulmonary Cell Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Our research efforts are directed toward investigating the life cycle and basic functions of various cell types lining pulmonary airways, in a well-defined in vitro condition. Such a culture offers a model system to investigate the mechanism of cell differentiation, as well as the repair of injury and the pathological response of cells to environmental toxic substances. Primary cultures from rabbit, rat, hamster, and mouse tracheal epithelium have been established. Experiments are carried out to determine: 1) the life span of normal epithelial cells in culture, 2) the origin of cultured cells in tracheal epithelium, 3) the fates of various cell types in culture, and 4) differentiated properties. We have observed that rat tracheal epithelial cells lose their differentiated properties immediately after plating, but regain their differentiated function. after plating in denuded tracheal graft. Furthermore, rabbit tracheal epithelial cells maintain some properties of mucus production and response to rentinoid and, therefore, the in vitro tracheal epithelial cultures can be used as a model system to study mucus cell differentiation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Tracheal epithelium was exposed to 0.5 to 1.0% of pronase solution at 4°C for overnight. Epithelial cells were than isolated for tracheal lumen by flusing medium through. Transmission and scanning EM were used to assess differentiated structures of tracheal cells in culture. Column chromatography on Sepharose 2B or 6B was used to separate mucus-like glycoprotein from bulk of glycoprotein.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Long term cultivation of tracheal epithelial cells in a defined, hormone-supplemented medium.

We have demonstrated that the continuous multiplication of rabbit tracheal epithelial cells could be achieved in a defined, hormone-supplemented medium as opposed to the conventional medium containing 10-20% fetal govine serum. This result has been extended to tracheal epithelial cells from other animals, rat, mouse and hamster. In addition to hormone supplements, a collagen-coated substratum was found to enhance cell growth and survival in vitro for most tracheal epithelial cells except those from rabbit. The standard culture medium for tracheal cells contained 50% 3T3 fibroblast-conditioned medium in Ham's F12 nutrient medium supplemented with insulin, hydrocortisone, transferrin, and bovine hypothalamus extract. Epidermal growth factor was found necessary for rabbit tracheal epithelial cultures. Using this defined culture system, we began to study the life cycle and differentiated functions of tracheal epithelial cells. It was shown that epithelial cells had limited life span. In case of adult rabbit cells, a 25 to 35 population doublings was observed.

2. Characterization of tracheal epithelial cells in cultures.

The purpose of this research is to determine the origin of cells grown in culture and to elucidate processes involved in cell dedifferentiation which may help to understand the nature of cell differentiation. We have observed a preferential attachment of in vivo proliferative cells (labeled by 3H-thymidine) over those non proliferative cells. However, a significant fraction of attached cells were either ciliated (more than 10%) or mucus-secreting (more than 30%). Beginning at 16 hours of culture, progressive "dedifferentiation" occurred with internalization of cilia and mucus cells showed a progressive loss of granules. These changes were correlated with the onset of DNA synthesis in culture. We have observed that 95% of attached cells would replicate their DNA during the first 48 hours in culture. These results indicated that some of differentiated cells, including possible cilia cells, were able to change from non-proliferative to proliferative stage in culture. This change was apparently paralleled with the loss of cell differentiated function. Furthermore, re-differentiation of these undifferentiated cells could be achieved in denuded tracheal grafts. Studies are now in progress to determine factors and mechanisms involved in the regulation of differentiation as well as dedifferentiaiton.

3. In vitro synthesis of mucus-like glycoprotein in tracheal epithelial cultures.

Primary and subsequently passage cultures of rabbit tracheal epithelial cells produced a large quantity of mucus-like glycoprotein. Chemical properties of these glycoproteins were shown mucus like nature: such as 1) large molecular weight, 2) sensitive to NaOH hydrolysis, 3) resistant to hyaluronidase digestion, and 4) amino acid composition. We are attempting to produce anti-serum of these in vitro mucus-like glycoproteins. The antibody could be useful for the identification the origin of these products in tracheal epithelium and the quantitation in vitro.

Retinoic acid, as low as 0.1 μg/ml, enhanced the secretion of these glycoproteins, yet there was no effect at this level on cell growth, and cell attachment. Analysis of the secreted product in Sephaose 6B, revealed that the stimulation was more significant in the mucus-like glycoprotein fraction, although simulation of the production of lower molecular weight of glycoprotein was also observed in retinoic acid treated cultures. These results suggest that in vitro tracheal epithelial cultures will be useful for study mucus-cell differentiation.

SIGNIFICANT TO BIOMECIAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The advantage of studying a specific cell type in culture is apparent: the number of variables to be controlled is markedly reduced. With more than 40 different cell types with a diversity of metabolic activity in the lung, the necessity to use in vitro system to study pulmonary function and environment-related problems is quite apparent.

We have shown that normal tracheal epithelial cells could be cultivated in vitro. A detail characterization od cultured cells revealed that epithelial cells could lose and regain their differentiated function. A similiar phenomenon may be existed in vivo when tracheal epithelium is exposed to a different environment. Such a change in epithelial properties will affect various pulmonary functions; such as pulmonary defense, drug metabolism and mucociliary function. Furthermore, we have shown that tracheal epithelial cultures can be used as a model system to study mucus cell differentiation.

PUBLICATIONS

Wu, R. and Groelke, J. W.: Characterization of Mucus-like Glycoprotein Secreted by Primary Rabbit Tracheal Epithelial Cells Grown in Serum-free Hormone-supplemented Medium. In vitro. 17: 245, 1981.

Wu, R., Nettesheim, P. and Change, L.: Early Changes of Rat Tracheal Epithelial Cells in Primary Culture. In vitro. 17: 238, 1981.

Wu, R., Wolfe, R. A. and Sato, G. H.: Distinctive Effects of Hydrocortisone on the Modulation of EGF binding and Cell Growth in HeLa Cells Grown in Defined Medium. J. Cell. Physiol., 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25007-03 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Particle Translocation in Various Cell Types and Anatomic Regions of the Lung

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

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Other:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

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 (200 words or less - underline keywords)

Inhalation of asbestos fibers and silica crystals causes debilitating lung disease in humans. We have established animal models to study several basic pathogenetic mechanisms. For asbestos, rats were exposed to an aerosol of chrysotile fibers for one hour. Inhaled fibers were deposited initially on alveolar duct bifurcations. Five hours after initial deposition, significant numbers of asbestos fibers were cleared from alveolar surfaces. These fibers were translocated to alveolar macrophages, to alveolar epithelial cells, as well as to basement membranes, interstitial cells and connective tissue. One day after exposure, asbestos laden macrophages significantly increased tissue volume in the region of alveolar duct bifurcations where the asbestos originally was deposited, thus forming the initial lesions of asbestosis. Similarly, the number of aerosolized crystalline silica particles on alveolar surfaces was significantly reduced 6 hrs after exposure, and by 24 hrs post-exposure, alveolar particles were rare. Silica h and by 24 hrs post-exposure, alveolar particles were rare. Silica had been translocated to macrophages and epithelial cells. Interestingly, 65% of the macrophages lavaged from the lungs of exposed animals contained silica crystals by six hours post-exposure. This percentage was maintained through a 24 day postexposure period, but the number of intracellular particles decreased over time.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED:

Asbestosis Model: Fischer 344 rats were restrained in cylinders during a one-hour nose-only exposure to 3.3 mg per m^3 (respirable mass) of chrysotile asbestos). Groups of four animals were sacrificed immediately after exposure. Additional animals were studied at 5 hrs. and 1, 4, and 8 days after exposure. The lungs were fixed by vascular perfusion to prevent disturbance of the inhaled asbestos fibers on airway and alveolar surfaces. This was accomplished by perfusing the right ventricle and pulmonary artery with Karnovsky's fixative after clamping the trachea and cutting the renal artery. After 10 minutes of perfusion, the lungs are removed *in toto* from the chest cavity and dissected for light, scanning, and transmission electron microscopy.

Silicosis Model: Fischer 344 rats in open cages were exposed to an aerosol of crystalline alpha quartz for 3 hrs. at a respirable mass of 90 mg per m^3. Animals were sacrificed immediately after exposure and at 6, 12, 24, 48 and 72 hrs. postexposure. Additional rats were killed at 7, 10, 14 and 24 days after exposure. Groups of animals were vascular perfused and tissue prepared for microscopy as described above. Additional groups of rats exposed to silica, as well as sham-exposed controls were anaesthetized at the time periods indicated above and the lungs lavaged with a Ca and Mg-free buffer. The lavaged cells were studied to assess the following physiologic and functional parameters: (1) cell number, differential and viability, (2) oxygen consumption and phagocytic potential, and (3) percentage of lavaged macrophages which contain crystalline silica.

MAJOR FINDINGS AND PROPOSED COURSE: We have reported that the majority of inhaled asbestos fibers which pass through the bronchioles are deposited initially at bifurcations of alveolar ducts. These fibers are translocated to alveolar macrophages, Type I alveolar epithelial cells, as well as to underlying basement membranes and connective tissue. Recently, using scanning electron microscopy, we determined that a significant number of fibers was cleared from the surface of bifurcations during the first five hours after exposure. By 24 hrs. after exposure, accumulations of asbestos-laden macrophages significantly increased tissue volume in the region of alveolar duct bifurcations where the fibers initially were deposited. We propose that this cellular reaction is an early and important event in the pathogenesis of asbestosis.

Ongoing studies are designed to determine the mechanisms by which inhaled asbestos fibers are transported through the alveolar epithelium to the interstitium as well as to capillary endothelial cells and lumena. Our hypothesis is that actin containing microfilaments polymerize around asbestos in the cytoplasm and the fibers are drawn toward the interstitium from where they may be cleared by lymphatic or vascular channels. Additional studies are focused on the functional and physiologic characteristics of macrophage populations from asbestos-exposed animals.

The silica exposed animals exhibited numerous particles scattered across alveolar surfaces. Immediately after exposure we determined that there were .015 ± .005 (mean ± SD) particles per micron squared of alveolar duct surface. Six hours

after exposure the number was significantly reduced to 0.008 ± .001; and after 24 hrs., the number of particles was reduced further to .003 ± .001. The closer to the terminal bronchiole, the more silica particles there were per micron squared of alveolar duct surface. Macrophages played a major role in clearing silica particles from alveolar surfaces. Using scanning electron microscopy in concert with X-ray energy spectrometry, we determined that immediately after exposure about 20% of the macrophages lavaged from exposed animals contained silica. Six hrs. after exposure, the percentage of silica positive cells increased to about 60% and this level was maintained through the 24 day period studied. Also, we determined that the number of particles per cell decreased gradually over time, and by 24 days, the number of particles per cell was not significantly different than at the immediately post-exposure sacrifice time. These findings demonstrate a rather long residence time for inhaled particulates on the alveolar surface and the continuing role of the macrophage in translocating particles from alveolar surfaces.

Ongoing studies of silica inhalation are being carried out to determine how the toxic nature of silica effects the clearance kinetics of alveolar macrophages and how particle toxicity effects macrophage populations in situ on alveolar surfaces.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Asbestos and silica are toxic particles which cause severe lung disease. The basic mechanisms which lead to clinically evident disease largely are unknown. Furthering an understanding of these mechanisms may provide a scientific basis for dust control and therapeutic intervention.

PUBLICATIONS

Brody, A. R., Roe, M. W., Evans, J. N. and Davis, G. S.: Use of backscattered electron imaging to quantify the distribution of inhaled crystalline silica. Scan. Elect. Mic. 3: 301, 1980.

Brody, A. R., Hill, L. H., Adkins, B. and O'Connor, R. W.: Chrysotile asbestos inhalation in rats: deposition pattern, reaction of alveolar epithelium and pulmonary macrophages. Amer. Rev. of Resp. Dis. (in press) 1981.

Brody, A. R. and Davis, G. S.: The alveolar macrophage: modifying factors and events in pulmonary toxicity. In: Mechanisms in Toxicity (Nettesheim, P. and Witschi, H., Eds.) CRC Uniscience Series, CRC Press, Inc. Boca Raton, Florida (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25008-03 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Elemental Analysis of Asbestiform Minerals

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

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Other:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Chrysotile is the most cytotoxic and most commonly used asbestiform mineral. We use this asbestos type in inhalation and in vitro studies. There is good evidence that the elemental nature of this asbestos plays an important role in its cytotoxic potential. Previously, we determined the dehydrating agents and fixatives that alter the elemental content of chrysotile, and we showed that elemental changes take place in the lungs of rats that have inhaled the fibers. Now we have shown that only magnesium ions are translocated from acid-treated chrysotile fibers and no significant silicon leaching can be detected by electron beam techniques. In addition, we have initiated studies to determine in which anatomic compartment(s) of the lung the elemental content of chrysotile asbestos can be altered. Data has been collected by electron microscopic and associated X-ray energy spectrometric techniques. Anatomic regions containing inhaled asbestos are the airway, alveolar and capillary lumena, airway and alveolar epithelial cells, pulmonary macrophages and interstitial cells and connective tissue. No elemental changes (compared to the control asbestos) have been detected through the eight day post-exposure period studied Longer time periods after inhalation are under investigation.

PROJECT DESCRIPTION

METHODS EMPLOYED:

In vitro: The asbestos used in this study was commercially derived chrysotile (Johns-Manville, Grade 4). Previous studies from this laboratory have shown the Mg to Si ratio to be 0.71 ± .04 (mean ± 1SD) as determined by X-ray energy spectrometry (XES). After treatment with 1N HCl for 16 hrs, this asbestos exhibits an XES Mg to Si ratio of 0.44 ± .04. These two populations of fibers are compared in the present study. We chose to look at fibers with diameters less than or equal to 1.0 micrometer since these were most abundant in our sample and are of primary interest for ongoing studies in the Laboratory. Dry fibers from both untreated and acid-treated samples were spread on carbon planchettes. Individual fibers were analyzed in a JEOL 35 C scanning electron microscope with attached X-ray energy (Kevex 7000) and wavelength spectrometers. Subsequent analytical procedures were identical for both the population of untreated fibers and the asbestos that had been treated with 1N HCl.

X-ray wavelength spectrometry (XWS) was used to measure the relative amounts of Mg and Si in individual asbestos fibers. The asbestos on a carbon substrate is placed in the SEM and a secondary electron image of an asbestos fiber is obtained. The electron beam is moved along the long axis of the fiber, and in the spot mode Mg and Si are measured independently for a given fiber. The results are expressed as Mg or Si X-ray counts per second. The X-rays characteristic of Mg and Si were identified and collected from the individual fibers. Mg and Si standards were used to maximize the collection angle of the diffracting crystal, and this angle was maximized for both elements on individual fibers as well. At least 4 fibers of a given diameter in both the treated and untreated fiber populations were analyzed, and at least 3 spots were analyzed for both Mg and Si along the long axis of each fiber.

In vivo: White rats were exposed to chrysotile asbestos for one hour, and three animals were sacrificed immediately after exposure, at 5, 24, and 48 hrs., and at 4 and 8 days post-exposure. The lungs were fixed by perfusion of the vasculature with Karnovsky's solution and prepared for scanning and transmission electron microscopy by conventional techniques. Thin sections of lung tissue ($\sim$ 500 A) were picked up on copper grids which were placed in a graphite specimen carrier prior to placing in a JEOL 100 CX electron microscope equipped with a computer-based X-ray energy spectrometer. Asbestos fibers in various anatomic locations were identified in the electron microscope and analyzed for Mg and Si content.

MAJOR FINDINGS AND PROPOSED COURSE:

In vitro: Little variation was observed among Mg values obtained by XWS from spot analyses along individual fibers. Likewise, there was little variation among the Si values from an individual fiber. Mg to Si ratios have been used to characterize untreated and acid-treated fiber populations. To determine if a change is occurring in one or both of the elements, we initially demonstrated a mass effect by XWS. As expected, the number of Mg and Si X-ray counts increased along with fiber diameter. Regression lines corresponding to numbers of Mg and Si X-rays versus fiber diameter exhibit linear correlation coefficients very close to 1.0. The data

were compared by least squares analysis, and a t-test of the raw data showed that acid treatment significantly effected the Mg values ($p=<.005$). Si values were not significantly changed by acid treatment. These findings were further verified where lines were calculated with slopes representing ratios of Mg (acid-treated) to Mg (untreated) and Si (acid-treated) to Si (untreated). The slope (ratio) of 1.04, suggested that no Si ions measurable X-ray wavelength analysis have been leached from chrysotile asbestos by treatment in 1N HCl. In contrast, the slope of 0.73 for the Mg:Mg ratio is significantly different from 1.0 ($p=<.005$) and demonstrates that Mg has been leached from the acid-treated fibers.

In vivo: Weight percents of Mg and Si in individual asbestos fibers were calculated by comparing the elemental content of the inhaled asbestos with 100% standards of Mg and Si. First, we determined that control asbestos prior to inhalation and after embedding contained a magnesium weight percent (wt. pct.) of 48.72% ± 1.9 (N=24 fibers) and a silicon wt. pct. of 51.25 ± 1.9 (24 fibers). These values correlate very well with Mg and Si levels determined by conventional wet chemical methods, i.e. 50% Mg to 50% Si. After treating these control fibers with 1N HCl for 4 hrs. the wt. pct. of Mg went down to 34.70% ± 10 (N=18). This exercise was necessary to determine instrument capabilities and accuracy while measuring elemental wt. pct. in plastic-embedded thin-sectioned asbestos fibers. Inhaled fibers thin-sectioned in the rat lungs showed no significant changes in any of the anatomic compartments studied through the eight-day sacrifice period, e.g. fibers in alveolar macrophages contained 46.05% ± 2.1% Mg (as described above, significant Si does not translocate from chrysotile fibers). Longer post-exposure periods currently are being studied to determine the initial events and anatomic location of alterations in inhaled asbestos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The relationship of chrysotile's physical and chemical characteristics to its cytotoxic potential has received limited attention. We and others have shown that the elemental content of chrysotile can be altered by a variety of reagents and by interactions within as yet unknown constituents of human and animal lungs. Currently, there is debate on whether it is the Mg or Si ions which are the primary elemental contributors to asbestos-induced cytotoxicity. Thus, it is important to know which elements have been altered in order to understand ongoing mechanisms of cytotoxicity. We plan to determine the time-related sequence of events and the lung anatomic compartments in which these elemental alterations take place.

PUBLICATIONS

Brody, A. R.: Elemental content of chrysotile asbestos: Mg/Si ratios in vitro and in situ after fiber inhalation. J. of Environ. Pathol. and Toxicol. 4: 5-12, 1981.

Hertzberg, M. A., and Brody, A. R.: Magnesium leaching and silicon stability in chrysotile asbestos fibers. Environ. Res. (in press) 1981.

Brody, A. R. and DeNee, P. B.: Biological interaction of inorganic particles in the lung. In: Critical Reviews in Environmental Control, (Straub, C. P., Ed.) CRC Uniscience Series, CRC Press, Inc., Boca Raton, Florida (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25009-02 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cell Membrane Interactions with Chrysotile Asbestos

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

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Other:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
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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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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Positively charged chrysotile asbestos fibers adhere to negatively charged sialoglycoproteins normally found on cell membranes, purportedly causing lysis of the cells. Previously, we labeled sialoglycoproteins on red cell membranes with a marker visible by electron microscopy (a lectin, wheat germ agglutinin (WGA) - conjugated to gold chloride (Au) spheres). Normally the Au-WGA complexes are distributed evenly on red cell surfaces. Our quantitative data show that the number of Au-WGA markers is significantly decreased on the surfaces of red cells which have been distorted during interaction with asbestos. WGA binds specifically to N-acetyl glucosamine (NAG), but non-specifically to N-acetyl neuraminic acid (NANA, a sialic acid). Thus, we pre-treated cells with neuraminidase and found that they were protected from distortion by asbestos. This corresponds with the original hypothesis that positively charged chrysotile asbestos binds to negatively charged sialic acid groups. Loss of the Au-WGA label subsequent to asbestos interaction suggests that this binding causes a depletion or redistribution of at least two sialoglycoprotein components (NAG and NANA). This is coincident with marked cell distortion and may be a basic mechanism through which chrysotile asbestos exerts its toxic effects upon a variety of cell

PROJECT DESCRIPTION

METHODS EMPLOYED: Red blood cells (RBC) from rats were collected by cardiac puncture and diluted in 0.85% saline to a 2% solution. All control and chrysotile asbestos ($MgO.SiO_2.H_2O$) reactions were carried out at 40°C. Gold spheres measuring .05 μm in diameter were prepared by refluxation of AuCl salts in the presence of Na citrate. The spheres were complexed with wheat germ agglutinen (WGA) using bovine serum albumin and glutaraldehyde according to established methods. The following reactions were carried out in tris-buffered saline on at least three separate occasions: (1) RBC's + asbestos (4 hr. reactions), (2) RBC's + Auspheres, (3) RBC's + AuWGA, (4) RBC's + Asb. + Au-WGA, (5) RBC's + WGA + Au-Wga, (6) RBC's + Neuraminidase, (7) RBC's + Neur. + Asb., (8) RBC's + Neur. + Au-WGA, (9) RBC's + WGA + Asb., (10) RBC's + Mg-leached asbestos. All specimens were fixed in 1.0% Karnovsky's following the 4 hr. reactions and critical point dried from ethanol for scanning electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Small asbestos fibers adhered to red cell membranes and during the four hour reaction time caused marked distortion of 80% of the cells. The distorted cells, but not normal appearing cells, exhibited decreased numbers of Au-WGA spheres per unit area of red cell surface: control = 21 ± 8 (Mean ± 1SD) (55 observations), asbestos-treated = 6 ± 0.5 (N=60). Pretreating the cells with WGA prevented labeling with Au-WGA, but pretreatment with neuraminidase only decreased the number of Au-WGA spheres, demonstrating the nonspecific binding to sialic acid. Most important, pretreatment of cells with neuraminidase protected them from distortion by asbestos. Pretreatment with WGA conferred only slight protection to the red cells. This illustrates the attraction of chrysotile asbestos for negatively charged sialic acid. Moreover, after removing a controlled amount of positively charged Mg from the asbestos fibers by acid treatment, there was decreased cell-asbestos binding and decreased cell distortion.

We have shown that chrysotile asbestos binds to red blood cells and causes membrane distortion. This reaction correlates with a decrease or masking of constituents within membrane sialoglycoprotein complexes. The asbestos appears to bind specifically to the sialic acid of these complexes. We propose to determine whether or not this binding and subsequent cell distortion is a significant event which leads to cytolysis. This will be studied by intracellular analysis of ion pools (Na, k, Ca) using electron beam microanalytical techniques on normal and asbestos-damaged cells. Subsequently, we will extend these studies to pulmonary components such as macrophages and epithelial cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Asbestos may exert its toxic effects on a variety of cell types through its interaction with membrane components. Elucidation of the basic mechanisms of this toxicity would be a significant step forward in understanding the initial pathogenetic patterns of asbestos-induced lung disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25011-02 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Prostaglandins in Tumor Promotion

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

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
Others:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	E. E. Sisskin	Staff Fellow	LPFT	NIEHS
	B. E. Tainer	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group, Environmental Carcinogenesis Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.25	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The goal of this project is to determine whether tumor promotion by phorbol esters (TPA) proceeds via the release of arachidonic acid (AA) and the subsequent synthesis of prostaglandins (PG's) and hydroxy fatty acid (HFA). We have chosen the mouse embryo fibroblast (10T 1/2) as a model system. TPA caused a dose-dependent release of arachidonic acid and subsequent conversion to PG-HFA's. The major arachidonic acid metabolite produced by the TPA addition to cells was an unidentified HFA. Reported inhibitors of the various metabolic pathways of AA metabolism were studied with respect to potency and selectivity. Several inhibitors were selected and are now currently being studied for effects on TPA-induced cell transformation. We intend, hopefully to correlate effects on promotion with effects on AA metabolism.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Various fibroblasts were grown in culture under normal conditions. Mouse (10T 1/2), hamster embryo (SHE) fibroblasts and a transformed derivative of HE (BP6T) were used in these studies. Cells were labeled with ^{3}H-Arachidonic Acid (AA) by growing cells for 24 hours in media containing ^{3}H-AA. After washing the cells to remove unreacted AA, the cells were exposed to the tumor promoter 12-O-tetradecanoyl phorbol 13-acetate (TPA) for varying lengths of time and at various concentrations. Release of AA and the formation of PG's, TX's, and HFA's were estimated by HPLC analysis.

MAJOR FINDINGS AND PROPOSED COURSE: The mouse embryo fibroblast (10T 1/2) was chosen as a model system for studying the correlation between prostaglandin (PG) formation from released arachidonic acid (AA) and tumor promotion by the phorbol esters (TPA). This fibroblast cell line was chosen based on the high amounts of PG's made by these cells and the ability of TPA to promote 10T 1/2 cell transformation. Production of PG's by these cells was characterized; the amount of PG produced was highly dependent on cell density.

On incubation of ^{3}H-AA with 10T1/2 cells, the majority of the radioactivity was incorporated into phospholipid. The addition of TPA to cells produced a dose-dependent release of ^{3}H-AA and subsequent formation of PG's and HFA's. Using this method, the effect of various inhibitors was investigated. The inhibitors were not very specific, for example ETYA reported to inhibit both cycloxygenase and lipoxygenase also inhibited the release of AA from lipids. Based on this information, we selected the following inhibitors to be studied in promotion assays: (1) Dexamethasone and hydrocortisone inhibit release of AA, (2) Indomethacin-inhibits PG formation, (3) Benoxyprofen inhibits the formation of PG's and HFA without altering AA release. These chemicals are currently being studied for their effect on benzo(α)pyrene-induced cell transformation by TPA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of non-carcinogenic chemicals increase the number of tumors observed and decreases the time before tumors appear after exposure to a carcinogen. The mechanism responsible for the promotion of tumors is not known. Recent reports have shown that a number of tumor promoters release arachidonic acid and stimulate PG production. At the present time, there is no simple adequate test to determine whether a chemical is or is not a promoter. If this hypothesis is correct, then a test system for determining promotion potential of a chemical could be developed. Determination of the mechanism of action for tumor promoters would significantly add to our understanding of carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25012-02 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Lung as an Endocrine Organ Controlling Intravascular Thrombosis

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

PI:	T. Eling	Head, PG	LPFT	NIEHS
Others:	J. Boyd	Technician	LPFT	NIEHS
	R. Korbut	Visiting Associate	LPFT	NIEHS
	B. Tainer	Biologist	LPFT	NIEHS
	R. Warnock	Chemist	LPFT	NIEHS
	J. Shirley	Temp. Technician	LPFT	NIEHS

COOPERATING UNITS (if any)

None

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:
3.2	1.0	2.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The goal of this study is to determine the role of pulmonary metabolism of essential fatty acids, e.g., arachidonic acid in the etiology of intra-arterial thrombosis. This study will determine the factors controlling production of prostaglandins and thromboxanes by pulmonary tissue and vascular endothelium. We have found that respiration rate influence the pulmonary production of PGI_2 and TXA_2. Increasing the respiration rate preferentially stimulates PGI_2 biosynthesis. The presence of platelets or platelet membranes in the vascular bed acts as a stimulator of membrane phospholipase liberating arachidonic acid resulting in an increase release of PGI_2 from the lung. Using in vitro systems, we have studied the role of peroxidase in control of PGI_2 biosynthesis. Chemicals that stimulate the peroxidase reduce levels of hydroperoxides that inhibit PGI_2 biosynthesis and result in a stimulation of PGI_2 production.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandins and thromboxanes were measured by radioimmunoassay, thin-layer radiochromatography and high performance liquid chromatography with subsequent liquid scintillation counting. Platelet aggregability was measured by a "platelet-aggregation photospectrometer." The metabolism of essential fatty acids as well as PG-transport studies were performed 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 vasculature and platelets was studied using the isolated perfused rat, guinea pig and rabbit lung.

MAJOR FINDINGS AND PROPOSED COURSE: Using the isolated perfused rat lung, we have studied the effect of respiration on the release of PGI_2 and TXA_2. At normal respiration rates, both PGI_2 and TXA_2 were detected in the perfusate at ratio of 5:1. Increasing the rate of respiration from 50 to 100 breaths/min increased the release of both PGI_2 and TXA_2 3- to 4-fold. The ratio of PGI_2 to TXA_2 increase from 5:1 to 12:1 indicating a preferential stimulation of PGI_2 biosynthesis. These findings suggest that rat lung generates PGI_2 predominantly and that respiratory movement can influence the generation of PGI_2 and TXA_2. We have also examined the effects of platelet presence in the vascular bed on the generation of PGI_2 and TXA_2 by the lung. As the concentration of platelets (or platelet membranes) increased, the amount of PGI_2 and TXA_2 increased 5 to 6-fold. Treatment of the lung with mepracine, a phospholipase A inhibitor, reduced the platelet stimulated release indicating that platelets are acting by release endogenous AA from phospholipid pools. Treatment of endothelial cells with platelets or membranes did not stimulate PGI_2 production. The results suggest that platelet stimulated release of PGI_2 and TXA_2 occurs via mechanical stimulation of phospholipase A_2, liberating arachidonic acid.

A primary culture of pig aortic endothelial cell was developed to study the biosynthesis of PGI_2 and HFA. We found that these cells make large amounts of PGI_2 but surprisingly the major AA metabolite was a HFA. This unidentified HFA appear to be a dihydroxy FA and may be 5,12-diHETE the end-product of leukotriene (LT) formation. We have obtained authentic samples of these diHETE and LT, developed a system for analysis and are currently working on identification of the HFA.

A major focus was to study the factors which control the production of PGI_2. Vitamin K_3 was found to inhibit PGI_2 biosynthesis in a concentration dependent manner. At 10^{-6}M, HPLC analysis of the AA metabolites produced by these cells revealed that K_3 specifically inhibit PGI_2 synthetase. No or little effect was observed on the formation of the other PGs or HFAs. A chemical, Bayer 674, and estradiol which have been reported to stimulate PGI_2 production were examined. Using ram seminal vesicles as a source of PGI_2 synthetase, these chemicals stimulated PGI_2 but only at high AA concentration. Studies are in progress to demonstrate this finding in endothelial cells. From these experiments an hypothesis was developed which described a mechanism for the control of PGI_2 biosynthesis. Our major goal at this point is to obtain sufficient evidence to support our hypothesis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cerebral and coronary strokes (heart attacks) as a result of intra-arterial thrombosis consitute 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. The lung with its vascular bed and extensive endothelial lining apparently play a major, yet undetermined role in the control of platelet aggregation and thus thrombi formation. Changes in this endocrine function of pulmonary tissue by exposure to environmental agents may have an impact on the state of mechanisms that control the pulmonary formation of PGI_2 which should thereby significantly contribute to our understanding of and ability to prevent intra-arterial thrombosis.

PUBLICATIONS

Ali, A. E., Barrett, C. J., and Eling, T. E.: Prostaglandin and thromboxane production by vascular endothelial cells and fibroblasts. Prostaglandins 20: 667-679, 1980.

Boyd, J. and Eling, T. E.: Prostaglandin release and the interaction of platelets with pulmonary vasculature of rat and guinea pig. Thrombosis Research 19: 239-248, 1981.

Korbut, R., Boyd, J. and Eling, T. E.: Respiratory movements alter the generation of PCI_2 and TXA_2 in isolated rat lungs: Prostaglandins (in press) 1981.

Korbut, R., Boyd, J. and Eling, T. E.: PGI_2 and TXA_2 release from isolated lungs. Prostaglandins (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30085-04 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Induction of Macrophageal Lysosomal Enzymes By Chemicals

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

PI:	E. G. Tombropoulos	Research Chemist	PTG NIEHS
Other:	W. Gibson	Chemist	PTG NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Toxicology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The mechanisms involved in the regulation of production and release of alveolar macrophage lysosomal enzymes are important to inhalation toxicology because of their participation in the digestion of endocytosed foreign materials and their role in the induction of lung damage during their release.

The induction of lysosomal enzymes by morpholine in alveolar macrophages was examined. Morpholine produced a non-uniform response in the stimulation of lysosomal enzyme synthesis and its effect dependend on the sex of the animal from which the alveolar macrophages were obtained.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: In vivo experiments. Animals were exposed to 250 ppm morpholine vapors or to filtered room air for 6 hours daily. At the end of the exposure, the animals were sacrificed by injection of 125 mg pentobarbitol in the outer ear vein. Lung lavages from rabbits were obtained using septic techniques. The lavages were centrifuged at 600 g for 10 minutes and the macrophages isolated. In the in vitro experiments the macrophages were resuspended in the incubation medium. The incubating medium consisted of medium 199 with Hank's salts, 31 g penicillin and 50 mg streptomycin. The final cell concentration was 1.5 to 2 x 10^6 cells/ml. Five milliliters of the suspension was incubated in 25 cm^2 culture flasks. Three hours after the initial incubation, the majority of the cells adhered to the flask, the medium was poured off and new medium was added containing morpholine.

The previously reported induction of macrophageal lysosomal enzymes by morpholine was further investigated. The questions asked were: 1) Is the induction of lysosomal enzymes dose dependent? 2) What is the time period required for the initiation of the induction and is there a time limit after which no further induction occurs? and 3) What, if any, specific chemical group of morpholine is responsible for the induction of the lysosomal enzymes?

MAJOR FINDINGS AND PROPOSED COURSE: In morpholine concentrations of 0.4 to 5 mM, the degree of induction of α-mannosidase activity in vitro of macrophages derived from female animals was proportional to the concentration of morpholine in the incubation medium. In macrophages derived from male animals, the stimulation was dose dependent up to a concentration of 2.8mM morpholine; thereafter it levels off.

β-N-Acetylglucosaminidase activity increased up to a 1.0 μM concentration of morpholine. Higher concentrations of morpholine were inhibitory in comparison to the 1 mM concentration but they were above the control levels up to 5mM concentration. In macrophages derived from male animals, the stimulation of β-N-acetylglucosaminidase by morpholine reached its peak at 1 mM concentration and then leveled off.

Acid phosphatase activity increased up to 0.8 mM and thereafter leveled off in macrophages from female animals. In macrophages from male animals, the stimulation was of maximal at 3.2 mM concentration of morpholine before it leveled off.

In vivo it was found that five (six hour) exposures induced a maximum activity of α-mannosidase in alveolar macrophages of female animals. Further exposures up to 33 did not significantly increase this activity. In alveolar macrophages from male animals, although the maximum induction occurred during the first five inhalation exposures, this stimulation continued. After the 33rd exposure the stimulation was 30% higher than after the 5th exposure. The macrophages of male animals had a higher stimulation of α-mannosidase activity than those of the female animals after each exposure. β-N-acetylglucosaminidase exhibited the highest activity after the 5th exposure in the alveolar macrophages from males. Additional exposure did not increase the stimulation observed after the 5th dose. In alveolar macrophages from female animals, N-acetylglucosaminidase activity

started declining after its peak at the end of the 5th exposure but still was above control levels at the 33rd exposure.

Acid phosphatase activity increased after exposure to morpholine, arriving at its peak after the second exposure in male animals and after the 5th exposure in females. Thereafter acid phosphatase remained the same in macrophages from both sexes up to the 33rd exposure.

In vitro α-mannosidase activity in alveolar macrophages of both sexes increased from the 4th hour of incubation and was maximal after 10 hours; thereafter it remained at this level until at least the 26th hour.

β-N-acetylglucosaminidase increased steadily up to 18 hours of incubation in macrophages of female animals and of male animals, up to 4 hours. In both sexes after a peak stimulation, the enzymic activity leveled off.

Acid phosphatase activity increased steadily during a 24 hour period of incubation in macrophages derived from female; it leveled off after the 4th hour of incubation in macrophages from the males.

These experiments support the theory that the same stimulus produces different patterns of response by the macrophages oas to the induction of lysosomal enzyme studies and that the response is influenced by the sex of the animal from which the macrophages were derived. To investigate what chemical group, if any, is responsible for the induction of these lysosomal enzymes, the following chemicals similar in structure to morpholine were tested and compared to the enzyme inductions produced by morpholine.

1) Diethanolamine, a secondary amine from which morpholine is synthesized

2) Tetrahydropyran, similar in structure to morpholine having the ether group but not the amino group

3) Piperidine, having the amino group but not the ether

4) Dimethyl morpholine, having similar active groups to morpholine

Of the above compounds, the only one producing enzyme inductions similar to morpholine was dimethylmorpholine. It is concluded that both the ether and amino group are needed for the induction of α-mannosidase by morpholine.

Experiments are in progress to determine if straight chain compounds of similar molecular weight and chemical groups will induce lysosomal enzyme activity and therefore if the aromatic ring of morpholine is needed.

Also the effect of various hormones on enzyme inductions will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Lung macrophages provide an early pulmonary defense mechanism. The major defense is based on the ability of alveolar macrophages to endocytose extracellular material and to digest it though their lysosomal enzymes. These same enzymes used for digestion can be released under the influence of certain substances. The release of hydrolytic enzymes can account for extensive tissue damage during acute or chronic inflammation. Therefore, the mechanisms which regulate lysosomal enzyme production and release from alveolar macrophages are important to lung defense and discase studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80008-07 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biosynthesis, Release, Transport and Metabolism of Prostaglandins (PG's) and Hydroxy Fatty Acids (HFA) in Lung

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

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	R. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)
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LAB/BRANCH
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SECTION
Prostaglandin Group

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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 (200 words or less - underline keywords)

The goal of this project is to study the biosynthesis and inactivation of prostaglandins by the lung and other tissues. The effects of environmental agents on pulmonary transport and metabolism of prostaglandins are being investigated, and these effects are being related to pulmonary toxicity and damage. We have compared the formation of PGs from AA and PGH_2 by a pulmonary tissue obtained from humans and animals. Rat lung was more similar to human lung in the kind of PG's produced. Guinea pig lung was least like human lung in PG biosynthesis. The major AA metabolite from human lung was a HFA. Preliminary results indicate that the HFA may be 5,12-diHETE. In addition, we used ESP method to study the initial steps in the formation of PG's from AA.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG) thromboxane (TX) and hydroxy fatty acid (HFA) synthetase activity was measured in vitro using the microsomal protein from a variety of tissues and organs as an enzyme source. ^{14}C-arachidonic acid (AA) or prostaglandin endoperoxides were incubated at 37°C for various times and under several conditions. After incubation, the PG and TX were removed by solvent extraction, separated by thin-layer chromatography or high pressure liquid chromatography, and estimated by liquid scintillation techniques.

An isolated perfused rat, guinea pig, or rabbit lung was used to examine the uptake, metabolism, and efflux of prostaglandins (PG's) and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PG's and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography techniques. The unidirectional flux of PG into the lung was measured by extrapolation of the net uptake velocity of PG to zero time. PG and TX from either incubation mixtures or perfusate of lung were also measured by radioimmunoassays.

MAJOR FINDINGS AND PROPOSED COURSE: A rapid and simple method based on HPLC was developed for the separation of PG's. This system was expanded to permit further separation of HFA. We are currently developing a new method which will separate leukotrienes (LT). Using this system we have studied the formation of AA metabolites in a variety of test animals and in human lung. Using either AA or PGH_2, the human lung made PGI_2 and TXA_2 in approximate equal amounts. Rat lung was very similar to human lung in producing PG's from PGH_2. Guinea pig lung produced mainly TXA_2 and was least like human lung. Except for guinea pig lung, conversion of AA to PG's was very low and appears to be due to inactivation of cyclo-oxygenase during tissue preparation.

On incubation of human lung with AA the major product was an unknown HFA. The formation of this HFA was increased by the presence of Ca^{+2} and ionophore in the incubation system. HPLC analysis indicated that the HFA was not a mono-hydroxylated HFA nor 8,15-diHETE. The unknown may be 5,12-diHETE the degradation product of LT's. Further studies are in progress to determine the structure of this HFA. In addition, we are now studying the conversion of PGH_2 to PG's using isolated veins, arteries and bronchi from human lung to investigate regional difference in AA metabolism.

We have also studied the mechanisms involved in the oxidation of AA to PG's. Using ESR spin trapping techniques, we have identified a free radial involved in the oxidation of AA to PG's in ram seminal vesicle microsomes. Our data suggest that the trapped free radical is carbon-centered free radical at C-11 and is probably the first intermediate formed during the oxidation of arachidonic acid to PG's. We are currently using purified cyclo-oxygenase to further study the process.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PG's and HFA have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PG's across cell membranes. The lung is an important site for the synthesis and metabolism of PG's, alterations in the 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 PG and HFA but little is known of the cells responsible for biosynthesis. This information appears to be important for the elucidation of the role of PG in pulmonary disease.

PUBLICATIONS

Crutchley, D., Boyd, J., and Eling, T. E.: Potentiation of TXB release from guinea pig lung during anaphylaxis following exposure to 100% O_2. Amer. Rev. of Resp. Dis. 121: 695-699, 1980.

Eling, T., Warnock, R., Dick, D., and Tainer, B.: Separation of PG, TX, HFA and arachidonic acid by high pressure liquid chromatography. Prost. and Med. 5: 345-355, 1980.

Eling, T., Tainer, B., Ally, A. and Warnock, R.: Separation of arachidonic acid metabolites by HPLC. Method in Enzymology (in press) 1981.

Eling, T. and Ally, A.: Pulmonary biosynthesis and metabolism of prostaglandins and related substances. Bull. Europ. Physicol. Resp. (in press) 1981.

Mason, R., Kalyanaraman, B., Tainer, B., Eling, T. E.: A carbon centered free radical intermediate in prostaglandin synthetase oxidation of arachidonic acid. J. Biol. Chem. 255: 5019-5022, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80029-05 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Investigations of Human Pulmonary Diseases

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

PI:	G. E. R. Hook	Head, BPG	LPFT	NIEHS
Others:	L. B. Gilmore	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)
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Department of Pediatrics, Duke University

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SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Identification and characterization of disease-related pulmonary components could provide information concerning the disease process and also function as markers for the diagnosis and monitoring of the disease. Current attention has focused on the multilamellated myelin-like structures present in the alveoli and airways of patients with pulmonary alveolar proteinosis. The objectives of this study have been to elucidate the composition, structure and origins of this unusual material.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Bronchoalveolar lavage effluents from patients with pulmonary alveolar proteinosis were supplied by the Department of Pediatrics at Duke University Medical Center. These lavage effluents were obtained as a by-product of the therapy essential to the well being of the patients.

Insoluble materials were sedimented by centrifugation and then separated from the soluble phase for examination under the electron microscope following dehydration and embedding.

MAJOR FINDINGS AND PROPOSED COURSE: A major component of the insoluble materials present in lavage effluents from the lungs of patients with alveolar proteinosis consists of multi-lamellated myelinlike structures (MS) which appear to form extracellularly in the alveoli and airways of patients with this disease. The MS consist of trilaminated membranes whose thickness varies from 85 to 100 A. These membranes are separated from each other by a region of amorphous material between 150 A and 300 A wide. Digestion of the structures with proteases results in destruction of the amorphous material indicating that it must consist primarily of protein. Digestion of the structures with phospholipase C results in the destruction of the membranous constituents indicating that the membrane must be composed of phospholipid. The structures are also severely disrupted by chaotropic agents such as potassium thiocyanate and potassium iodide, suggesting that the structures are held together by hydrophobic interactions and not by covalent bonds.

These myelin-like structures will be further investigated as to their protein and lipid composition and compared with normal constituents of extracellular lining.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases except in the advanced states and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed.

Our studies indicate that the myelin-like material which accumulates in the lungs of patients with pulmonary alveolar proteinosis is closely related to tubular myelin as found in the lungs of normal humans. Apparently, in the lungs of patients the tubular myelin structure is assembled in an aberrant manner.

PUBLICATIONS

Nadeau, D., Reasor, M. J., and Hook, G. E. R.: Extracellular alkaline phosphatase from alveolar secretions of patients with pulmonary alveolar proteinosis. Can. J. Biochemistry 59: 290-300, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80032-08 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Composition and Origins of the Acellular Lining Layer of the Lung

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

PI:	G. E. R. Hook	Head, BPG	LPFT	NIEHS
Others:	J. W. Spalding	Research Biologist	LPFT	NIEHS
	L. B. Gilmore	Biologist	LPFT	NIEHS
	S. Douthit	Bio. Lab. Technician	LPFT	NIEHS

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Laboratory of Environment of Biophysics

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

INSTITUTE AND LOCATION
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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 (200 words or less - underline keywords)

The alveoli and distal airways of the lung are lined with an acellular layer of material which is essential for the maintenance of normal pulmonary functions such as gas exchange. The composition and origins of the acellular lining are being investigated. Current attention has been directed towards: (1) the biosynthesis and secretion of pulmonary surfactant and (2) the nature of the cytoplasmic organelles known as lamellar bodies. The objectives of this investigation are as follows: (1) to further develop methodology for the isolation of lamellar bodies from the lungs of rabbits (2) characterize the lamellar bodies according to phospholipid, protein and enzymic components, (3) to elucidate processes involved in the formation of the extracellular lining from secreted lamellar bodies.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Acellular lining material is obtained by lavaging the lung of rabbits via the trachea. Lamellar bodies from the cytoplasm of Type II cells are isolated on discontinuous sucrose gradients using differential centrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Lamellar bodies of the alveolar Type II cells are storage sites of pulmonary surfactant. These structures are secreted into the alveoli where the surface active phospholipids help stabilize the distal regions of the lungs.

Lamellar bodies were isolated from homogenized lungs of rabbits using a method developed in this Laboratory involving the use of discontinuous sucrose gradients. The isolated lamellar bodies appeared similar to those found in the cytoplasm of Type II cells insofar as their appearance under the electron microscope is concerned. The lamellar bodies retained their lamellae, perilamellar membrane and amorphous core. The structures were analyzed according to their phospholipid and fatty acid composition. Analysis of the structures by isopycnic centrifugation indicated that NADPH cytochrome C reductase detected in the lamellar bodies may be a component and not a microsomal contaminant as previously assumed.

The membranous components of the extracellular lining have been isolated from pulmonary lavage effluents from the rabbit. The fluidity characteristics of these membranes have been studied using electron paramagnetic resonance (EPR) and the probe 5-doxylmethyl stearate. These studies indicate that the extracellular membranes are highly fluid structures. However, compared with the extracellular membranes, the major constituent of those membranes dipalmitoylphosphatidylcholine (DPPC) is not very fluid at physiological temperatures. The fluidity of DPPC is modified considerably by the presence of other phospholipids.

These studies of lamellar bodies and pulmonary surfactant will continue. Lamellar bodies will be further characterized as to their enzymic components and ability to synthesize phospholipids. The fluidity properties of the membranous constituent of the extracellular lining will be further studied and attempts will be made to identify the active constituent responsible for modifying the fluidity of DPPC.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The acellular lining of the lung is vital for the maintenance of normal lung functions such as gas exchange. Inhaled toxicants such as the oxidant gases (e.g., ozone), particulate materials (e.g., silica) and chemicals (e.g., paraquat) appear to affect the acellular lining both qualitatively and quantitatively. The involvement of the acellular lining in the progression and mediation of some pulmonary diseases such as alveolar proteinosis appears certain. Unfortunately, the mechanisms which underlie these pulmonary diseases and agent-induced lung damage are not known. Elucidation of the biochemical processes which contribute to the formation of pulmonary surfactant and the acellular lining are a necessary step in the understanding of the disease process.

PUBLICATIONS

Bell, D. Y., Haseman, J. A., Spock, A., McLennan, G., and Hook, G. E. R.: Plasma proteins of the bronchoalveolar surface of the lungs of smokers and nonsmokers. Am. Rev. Resp. Dis. (in press) 1981.

Gilmore, L. B. and Hook, G. E. R.: Quantitation of proteins in polyacrylamide gels by the elution of Fast Green FCF. J. Biochem. Biophys. Methods (in press) 1981

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-05 LPFT

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neuroendocrine (Small Granule) Cells of the Lung

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

PI:	R. P. DiAugustine	Head, EG	LPFT	NIEHS
Others:	D. Vembu	Staff Fellow	LPFT	NIEHS
	I. Linnoila	Staff Fellow	LP	NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Endocrinology Group

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The epithelium of the lung of mammals is known to contain at least two principal classes of neuroendocrine-like, or small-granule cells, those which exist as solitary cells and those which exist in an organoid structure referred to as the neuroepithelial body. (1) In one study, quantitation of the solitary neuroendocrine cells of the cricoid epithelium of the guinea-pig larynx by electron microscopy revealed a homogeneous population consisting of 4-9% of the total epithelial cells. The neuroendocrine cells identified in the tracheal epithelium had the same ultrastructural properties as those observed in the cricoid. (2) Continued efforts to identify known polypeptide hormones in the trachea by immunocytochemical techniques revealed no positive cells. (3) Tracheal neuroendocrine cells, identified by argyrophilia in light microscopy, did not incorporate ^{3}H-thymidine into cell nuclei until approximately 3 days after administration of tracer, as demonstrated by autoradiography. This finding supports the notion that lung neuroendocrine cells are derived from a separate cell population. (4) The hyperplasia of lung neuroendocrine cells observed in hamsters treated with diethylnitrosamine appears to originate with cells of the neuroepithelial body or its progenitor.

PROJECT DESCRIPTION

METHODS EMPLOYED: General histological procedures were used for fixation and staining of tissue sections for light and electron microscopy. The Grimelius method was used to detect argyrophilic cells. Most of the antisera to various polypeptide hormones were obtained from rabbits following intermittent subcutaneous injections of the peptide coupled to albumin or hemocyanin; other antisera were obtained from the Pituitary Agency, individual investigators, or commercial laboratories. The immunoperoxidase-bridge technique was employed for immunohistochemical localization of polypeptide hormones. Details of the methods for dissociation of lung cells and cell culture are described in last year's report. Other methods used were autoradiography, radioimmunoassay, and gel filtration column chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: The present program consists of investigations of solitary neuroendocrine-like cells of the pulmonary epithelium and the response of these cells to a respiratory carcinogen, diethylnitrosamine (DEN).

Neuroendocrine-like Cells of the Normal Pulmonary Epithelium. This investigation essentially consists of two major parts. In the first, attempts were made to identify by electron microscopy neuroendocrine-like (NEC), or small-granule, cells in the upper respiratory tract of the guinea pig. We were especially interested in examining whether more than one subpopulation of NECs existed. In the present studies we confined our examinations to the epithelium of the cricoid region of the larynx and the tracheal epithelium. We also wanted to quantitate the number of NECs in this region of the airways.

We were readily able to identify NECs in the cricoid so this region was used initially for a systematic quantitative study. Solitary NECs were readily identified by the presence of unique small membrane-encapsulated granules (mean diam.: 131 ± 28 nm, SD). The ultrastructural characteristics of the NECs were homogeneous throughout the various regions of the cricoid sampled, suggesting a single population. Quantitation of NECs by electron microscopy was made by sequentially surveying cells along the basement membrane. NECs constituted 4-9% of the total cricoid epithelial cells in 2 days - to 7- week old guinea pigs. A representative quantitation is shown in Table 1. Only solitary NECs were observed and no specific concentration of these cells occurred in any region of the mucosal surface of the cricoid.

Animals treated with 5-hydroxytryptophan had cricoid cells with yellow fluorescence by the formaldehyde-induced fluorescence procedure, indicating that these NECs can synthesize serotonin.

Similar small-granule cells were identified by electron microscopy in the upper, middle, and lower regions of the trachea. Quantitation of these cells in the tracheal epithelium is now in progress for the epithelium covering the 3rd, 18th, and 34th cartilage rings.

The finding of a homogeneous population of cricoid NECs at the frequency reported provides a convenient system to assess their biological properties and eventually identify their humoral components. In one proposed study, we plan to examine

whether these NECs respond by degranulation or loss of serotonin to hyperpolarizing concentrations of $[K^+]$ as other NECs do. We speculate that a unique neuropeptide may be co-stored with serotonin in the lung solitary NECs.

TABLE 1

Quantitation of cricoid epithelial NECs in a 7 week-old guinea pig by electron microscopy. A 2 mm-thick section was made by slicing the cricoid transversely with a razor. The section was then divided in half by a section along the sagittal plane. Each of these halves was then divided equally by a longitudinal section. Finally, each of the resulting four cricoid pieces, as designated below, was examined for epithelial NECs.

Region examined	Total cells counted	Neuroendocrine cells
R1	70	5
L1	96	4
R2	51	2
L2	88	2
	305	13
		= 4% NEC

R = right
L = left
1 = ventral epithelial surface
2 = dorsal epithelial surface

In a second part of this work we exhaustively examined solitary NECs in the tracheal mucosa by histochemistry for immunolocalization of known polypeptide hormones. No positive cells could be identified in the epithelium. Other details are given in last year's report.

Cytokinetic analysis of the argyrophilic cells using ^{3}H-thymidine indicated no labeled nuclei (> 200 argyrophilic cells examined) after a 60 min-pulse. Autoradiographic analysis after 3 days did reveal labeled nuclei which suggests that the tracheal NECs derive from some separate population of epithelial cells (basal cells).

As with the cricoid epithelium, we propose to establish by EM whether a single population of NECs exists in the tracheal epithelium.

Proliferation of endocrine-like cells in lungs of hamsters treated with diethylnitrosamine (DEN). Details of the experimental design were covered in last year's report. The results of the study demonstrated *in vivo* hyperplasia of apparent neuroepithelial bodies and subsequent culture of neuroendocrine-like cells. Studies of the incorporation of ^{3}H-thymidine into NEBs of treated and untreated hamsters have been carried out independently by Dr. Ilona Linnoila, formerly in the Endocrinology Group and now with the National Cancer Institute.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of the biological properties of lung epithelial neuroendocrine cells and their humoral constituents should ultimately allow us to establish what function these cells have in the normal lung. Some lung-associated abnormalities may be initiated by improper levels of a regulatory peptide synthesized by a lung neuroendocrine cell. The experimental induction of lung neuroendocrine cell hyperplasia may indicate that certain lung tumors, namely bronchial carcinoid tumors and small cell carcinomas, may be preferentially formed by exposure to the nitrosamines in comparison to other classes of chemical carcinogens. Identification of lung-specific polypeptides should conceivably serve as a valuable plasma marker for early neoplastic responses of lung neuroendocrine cells.

PUBLICATIONS

Linnoila, R. E., Nettesheim, P. and DiAugustine, R. P.: Lung endocrine-like cells in hamsters treated with diethylnitrosamine: Alterations in vivo and in cell culture. Proc. Natl. Acad. Sci. (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80035-05 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Co-oxidation of Xenobiotics by the Prostaglandin and Thromboxane Synthetase

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

PI:	T. E. Eling	Head, PG	LPFT	NIEHS
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	J. Boyd	Temp. Technician	LPFT	NIEHS
	R. Mason	Research Chemist	LEB	NIEHS
	K. Sivarajah	IPA	LPFT	NIEHS
	D. Josephy	Visiting Fellow	LPFT	NIEHS
	R. Warnock	Technician	LPFT	NIEHS
	C. Motley	IPA	LEB	NIEHS
	J. Shirley	Temp. Technician	LPFT	NIEHS

COOPERATING UNITS (if any)
Drs. E. Zeiger and I. Robertson, Laboratory of Molecular Genetics; Dr. M. Anderson, Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.1	1.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The 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. BP-7, 8-diol metabolism was studied in 10T1/2 cells where the PG's and NADPH-dependent oxidation can be compared. Stimulation of PGS resulted in elevation of metabolism and higher cell transformation. These studies suggest a potentially important role for PGS in BP-induced cell transformation. In addition, we studied the metabolism of DES and acetoaminophen by PGS. Both chemicals are metabolized to reactive intermediates by PGS. The effect of a PGS inhibitor, aspirin on the development of BP-induced lung tumors in mice, is currently being studied.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues, such as guinea pig lung and ram seminal vesicles, were used to examine the cooxygenation of xenobiotics during prostaglandin synthesis. The BP-7,8-diol metabolites were isolated from the incubation medium by extraction and separation by HPLC. Prostaglandins (PG) and thromboxane (TX) products were also isolated and quantitated. Tissue cultures are used to examine the interaction of BP electrophilic metabolites produced by PG synthetase with DNA and to determine if this interaction is related to cell transformation. HPLC was used to isolate and characterize the metabolites formed.

MAJOR FINDINGS: The carcinogen, BP, 7,8-diol was oxidized by prostaglandin synthetase (PGS) to diol epoxide I. We have studied its oxidation in 10T1/2 cells measuring both metabolism and transformation. On stimulation of PG's biosynthesis by the addition of AA, a 2-4-fold stimulation of metabolism was observed. Indomethacin inhibited this stimulation. The major BP-diol metabolites produced by these cells was diol epoxide I and II. AA addition stimulated formation of only diol epoxide I. The stimulation of metabolism by AA was observed at low concentrations of BP. Cell transformation was measured at a variety of BP diol concentrations with and without AA and in the presence and absence of indomethacin. AA increased cell transformation 10-fold and this appeared to correlate with metabolism. Further studies measuring BP diol adducts to DNA are underway.

We have also studied cooxidation of BP-diol in a number of strains of mice and AHe/J were chosen as a model for studying BP induced lung tumor. The mice had high PGS-dependent oxidation of BP-diol activity compared to NADPH-dependent oxidation. Mice were treated with aspirin and the dose determined that completely inhibited PGS without effecting the cytochrome P-450 dependent oxidation. An experiment is underway to determine the effect of aspirin on the development of lung tumors. Animals were given 2 doses of BP and one dose of aspirin. In addition, in collaboration with Dr. Anderson, DNA adducts will be measured. This study will help determine the potential importance of cooxidation in development of BP induced lung tumors.

We have also investigated the oxidation of DES and acetoaminophen by PGS. Acetaminophen was oxidized to reactive intermediate by PGS located in kidney cortex. No NADPH-dependent oxidation was observed. Our data suggest that PGS dependent cooxidation of acetoaminophen may be related to kidney toxicity from chronic use of acetoaminophen.

We have also developed an Ames test using Ram seminal vesicles as source of PGS for the activity system. A number of chemicals have been studied. The bay region diol of benzanthracene and chrysene were activated. 2 AF (but not 2 AAF), β-naphthylamine, benzidine, several hair dyes were also activated by PGS.

Our proposed course is as follows: (1) to study a number of environmental chemicals that can be activated by the AA system and to determine the nature of the electrophilic metabolites formed; (2) to study AA-dependent oxidation in tissue cultures, to determine whether significant oxidation occurs in vivo and if so, whether the metabolites covalently bind to DNA and protein; and using tissue

cultures and in vitro mutagenesis systems to assess the significance of AA-dependent BP oxidation for cell transformation; (3) to study the mechanisms of AA-dependent oxidation of amines and hydrocarbons; (4) to examine the induction of PG synthesis by chemicals; (5) to study co-oxidation in vivo.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many xenobiotics are thought to exert their toxicity by means of reactive metabolites which are generated in vivo with very short half-lives. These metabolites react with tissue macromolecules to produce carcinogenesis, mutagenesis and teratogenesis. The prostaglandin synthetase system is found in most mammalian tissues and has particularly high levels of activity in the lung and kidney. Moreover, arachidonic acid can be released from its phospholipid storage sites by various types of stimulation, for example, irritation of lung tissue by inhaled pollutants. The subsequent metabolism of arachidonic acid by prostaglandin synthetase and the simultaneous co-oxygenation of xenobiotics could produce toxic metabolites.

PUBLICATIONS

Sivarajah, K., Mukhtar, H., and Eling, T. E: Arachidonic acid-dependent metabolism of ± trans-7,8-dihydroxy-7,8-dihydro benzo(a)pyrene to 7,10/8,9 tetrols. FEBS Letters 106: 17, 1979.

Sivarajah, K. and Eling, T. E.: Arachidonic acid-dependent metabolism of ± trans-7,8-dihydroxy-7,8-dihydro benzo(a)pyrene by pulmonary tissues. Comparison to NADPH-dependent metabolism. Cancer Research (in press) 1981.

Sivarajah, K., Lasker, J., Abou-Donia, M., and Eling, T. E.: Metabolism of N-alkyl compounds during the biosynthesis of prostaglandin. Mole. Pharm. (in press) 1981.

Lasker, J., Sivarajah, K, Mason, R., Kalanaraman, B., Abou-Donia, M., and Eling,T.: A free radical mechanism of prostaglandin-synthetase dependent aminepyrene demethylation. J. Biol. Clem. (in press) 1981.

Kalayanaraman, B., Tainer, B., Eling, T. and Mason, R. The free radical formed during the hydroperoxide-mediated deactivation of prostaglandin synthetase is enzyme derived. Biochem. Biophys. Acta. (in press) 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25015-01 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neuroendocrine cells in Rabbit Fetal Lung: A Model System for In-depth Study

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

PI:	K.S. Sonstegard	Senior Staff Fellow	LPFT	NIEHS
	R. P. DiAugustine	Research Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)
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Dept. of Neuropharmacology
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LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Cell Biology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Dispersed throughout lung airway mucosa are individual and innervated groups of cells with unique morphological, cytochemical and biochemical properties similar to neuroendocrine cells (NE) in the G.I. tract, carotid body, taste bud, thyroid and pituitary. The presence of serotonin, amine precursor uptake and decarboxylation enzyme mechanisms, acetyl cholinesterase, neuron specific enolase and at least one (bombesin) or more (enkephalins, calcitonin) neuropeptides suggests lung NE cells may have sensory or receptor mechanisms linked with respiratory centers in the CNS. These cells are difficult to locate in adult lung. However, 20 to 30% of all lung tumors are believed to be of NE cell origin possibly in response to environmental influence such as cigarette smoke, ozone, halo ethers, hypoxic conditions and diethylnitrosomine. Furthermore it is proposed that these cells may play an important role in early lung development since they appear to be the first cells to differentiate and are most numerous during the embryonic and fetal periods. Our objectives are to carry out systematic morphological, cytochemical and biochemical studies of NE cells in the fetal period. In order to do so, new systems of anlysis need to be developed.

PROJECT DESCRIPTION

The pulmonary neuroendocrine system is not easily amenable to experimental analysis because of cellular heterogeneity of the lung and complex intercellular relationships. Our aim is to develop new ways to examine both the structure and function of the lung NE system. Earlier studies suggest that NE cells form a greater proportion of the lung cell volume in prenatal lung and that growth and differentiation may distribute the NE cell population over a greater area. Morphological studies have shown single NE cells and neuroepithelial bodies (NEB) in 17 to 18 day fetal rabbit lung. Our plan is to carry out an in-depth morphological, cytochemical, and biochemical analysis of NE cells in late gestation (29 day) fetal rabbit lung in order to determine: 1) when NE cells first appear in developing lung, 2) the appearance of tryosine hydroxylase, the rate limiting enzyme in the synthesis of serotonin, dopamine and noradrenalin. 3) The ability of NE cells to synthesize and store serotonin, dopamine, and noradrenalin. 4) The presence of the amine precursor uptake and decarboxylation (APUD) enzyme mechanisms in NE cells. 5) The appearance of neuropeptides, i.e., bombesin, calcitonin, substance P, VIP etc. in NE cells. 6) The numbers of NE cells, in particular just prior to term and, 7) the effect of glucocorticoids, nerve growth factors, thyroid hormone and other factors on the differentiation, NE cell numbers and amine and peptide transmitters in embryonic and fetal lung NE cells.

METHODS EMPLOYED: New methods to examine both the structure and function of NE cells are being developed. We have previously identified at random NEB in near term fetal lung and lung explants by LM, TEM and SEM. New embedding and sectioning procedures allow us to locate NEB in unstained 20 μm sections and cut them from adjoining 60 μm sections for re-embedding. This facilitates a semi-quantitative recovery of NEB but not single NE cells from known airway locations. A systematic morphological and cytochemical evaluation of fetal rabbit NE cells is being carried out on fetal lungs of known gestational age. Histochemical and immunocytochemical procedures developed in this laboratory or by others demonstrate NE cells routinely in paraffin sections and, if the procedures lend themselves, in plastic sections and cytocentrifuged cell preparation (cytopreps) of dissociated fetal lung. Dissociation of lung into its component cells and concentration of isolated NE cells is another approach to permit the localization of morphological and biochemical constitutents and to examine key metabolic activities observed in whole tissue. We have developed a collagenase enzyme method to dissociate fetal lung tissues into repeatable yields (5.2×10^8) of cells with 87% viability. Isolated, single NE cells and NEB as intact, orgonoid structures retain their ultrastructural and metabolic integrity as demonstrated by HPLC identification of serotonin, its metabolite, 5HIAA, dopamine, and DOPAC as well as amine precursor uptake and decarboxylation mechanisms. Cellular and biochemical analysis of isolated cell fractions by unit gravity sedimentation demonstrate an enrichment of these cells.

MAJOR FINDINGS AND PROPOSED COURSE: Morphology: Corpuscular NEB are composed of several columnar cells (09 μm) organized at their apical surface by a juxtaluminal functional complex. The specialized surface area exposed to the bromine is approximately 314 μm and is covered by microvilli. The base of these corpuscular structures may indent into the subucosa. Primary cilia may extend from

individual cells in NEB. Perinuclear and basally-located neurosecretory granules may be at least 3 types depending on size, shape, electron density and presence of an electron-dense core and a clear halo inside the vesicle limiting membrane. It appeared that all cells in a NEB had the same type of granules but all NEB did not. Nerve axons particularly of the cholinergic type were located between NEB cells. Cells adjacent to and covering single NE cells and the majority of the NEB surface were undifferentiated with large amount of glycogen. We conclude fetal rabbit NEB and single NE cells are structurally and functionally differentiated before other cell types in immature airway epithelium.

Cytochemistry: Fetal NE cells and NEB are argentaffin-positive, argyrophilic and stain by localization of specific antibodies to serotonin and neuron specific enolase. Dopamine was demonstrated in NEB by paraformaldehyde-induced fluorescence.

Isolated lung cell system: A method was developed for the isolation from late fetal rabbit lung of single NE cells and NEB with an intact orgonoid structure and metabolic integrity. Cellular and biochemical analyses were made of collagenase-dissociated, viable, separated lung cell fractions. Isolated NEB and single NE cells were demonstrated by their argyrophilia. Fluorescence from paraformaldehyde condensation of intracellular cateocholamines and serotonin (5-HT) in isolated NE cells was weak to absent. Various histochemical methods also failed to demonstrate NE cells in cytocentrifuged cell preparations. Recovery of NEB varied in fractions separated from the monodispersed cell mode and having only clumps > four cells. From one to five NEB stained with silver in control cytocentrifuge preparations while as many as 10-15 intact NEB were demonstrated in enriched fractions. Isolated NEB revealed good ultrastructural preservation of cyto-plasmic dense-core vesicles and desmosomal connnections between NEB cells. Isolated NEB and NE cells appear to retain certain biochemical and metabolic properties similar to those of biogenic amine-containing tissues in situ. 5-HT, dopamine, and their metabolites, dihydroxyphenylacetic acid and 5-hydroxyindole acetic acid were measured by high performance liquid chromatography in mixed control cells and separated fractions. The distribution of biogenic amines, their metabolites and preferential demonstration of intact amine precursor uptake and decarboxylation enzyme systems as well as bombesin radioimmunoactivity in these fraction further substantiated enrichment of NE cells. These preparations provide a simplified system for metabolic studies and starting material for purification of NEB from developing lung.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
The structural complexities of whole lung make it difficult to investigate nonrespiratory functions of various pulmonary cells, particularly those which occur in relatively low numbers in the majority of the small airways and at branch points. Our specific aim is to develop in vitro model systems starting with whole lung and isolated airways. The importance of studies of this nature lies in the fact that neuroendocrine may be a target for several environmental factors which may be the cause of pulmonary injury and disease. Yet, our understanding of the function and life cycle of these cells is still very rudimentary.

PUBLICATIONS

Cutz, E., Chan, W., and Sonstegard, K.: Identification of neuroepithelial bodies in rabbit fetal lungs by scanning electron microscopy: A correlative light, transmission and scanning electron microscopic study. Ana. Rec. 92: 459-466, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25016-01 LPFT

PERIOD COVERED
October 1, 1980 to September 31, 1981

TITLE OF PROJECT (80 characters or less)

Identification and Isolation of a Peptide with Physalaemin-like Immunoreactivity from Mammalian Tissue.

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

PI:	Lawrence H. Lazarus	Research Chemist	LPFT	NIEHS
Other:	Richard P. DiAugustine	Research Chemist	LPFT	NIEHS
	Gloria D. Jahnke	Chemist	LPFT	NIEHS
	Oscar Hernandez	Research Fellow	LEC	NIEHS
	J. Ronald Hass	Research Chemist	LEC	NIEHS
	R. Ilona Linnoila	Research Fellow	NCI	NIH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We demonstrated the presence of physalaemin-like immunoreactivity in acid extracts of mammalian gastrointestinal tract and respiratory tract by use of an NH_2-terminal specific antiserum to the amphibian peptide physalaemin. By radioimmunoassay the highest concentration occurred in rabbit stomach (ca. 100 ng/g dry weight) with levels of 1-15 ng/g in pulmonary tissue from numerous species. Immunohistochemical staining revealed specific immunopositive cells in Brunner's gland, in the duodenum and ileum of guinea pig, and in the colon of rat. Nerve fibers were also identified in the esophageal sphincter of rat. Gel filtration studies indicated that the immunoreactivity had a Mr = 1700. This material was resistant to digestion by trypsin, α-chymotrypsin and CNBr. It eluted from alkylsilane columns in 35% methanol, whereas physalaemin appeared in 50% methanol. This material did not bind to Con-A. These data suggest that this immunoreactive material is a new neuropeptide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTON

METHODS EMPLOYED: All mammalian tissues were lyophilized prior to extraction in boiling formic acid-EDTA-DTT. The extracts were clarified by centrifugation and freeze-dried before radioimmunoassay (RIA). The RIA used antiserum raised in rabbits against a physalaemin-hemocyanin conjugate; the antiserum was specific for the NH_2-terminal region of physalaemin. Cross-reactivity was negligible (<0.0001%) with peptides lacking the common amino acid sequence Asp-Pro-Asn; even then, uperolein (a related tachykinin) cross-reacted only 2% in a nonparallel manner.

Immunohistochemistry used an improved lactoperoxidase bridge technique on p-benzoquinone-fixed tissues, which were embedded and sectioned in paraffin. Controls included preabsorption with non-immune serum and antiserum treated with excess antigen. Column chromatography employed Bio-gel P-4 columns equilibrated in and eluted with dilute formic acid. Column dimensions varied from 50 x 0.7 cm (analytical) to 95 x 1.5 cm (semi-preparative) to 50 x 4 cm (preparative). Alkylsilane (C_{18}) resin was a commercial product (Sep-Pak).

MAJOR FINDINGS AND PROPOSED COURSE: Immunoreactivity to physalaemin (PSLI) was found in the gut of guinea pig, neonatal and adult rat, rabbit and mouse. The highest levels are in the stomach of all mammals with rabbit containing more than all other species, ca. 100 ng/g dry weight. Furthermore, in rabbit, the concentration of PSLI in the antrum was higher than in the fundus, plylorus or corpus. In the respiratory tract, PSLI was found in the mucosal layer of trachea from canine, bovine, and porcine. In whole trachea analyses (rat, guinea pig, rabbit), rat contained 3-5 times more PSLI (ca. 13 ng/g) than any other species. The amount in rat lung was approximately 1/25 of that in trachea and even lower quantities were detected in the neonate (0.01 ng/g). Similar trace levels (0.08 ng/g wet weight) were found in a human oat-cell carcinoma propagated in nude mice. Preliminary data suggest PSLI exists in brain tissue as well, with the highest levels in the cerebellum and spinal cord. Tremendous variation occurred between assays with non-parallel responses frequently observed.

Immunohistochemical staining revealed the following: (1) specific staining in Brunner's gland; (2) immunostaining of endocrine-like cells in the duodenum and ileum of guinea pig and in the colon of rat; and (3) nerve fibers immunostained in the region of the esophageal-stomach sphincter. Preliminary findings also indicate the possibility of nerve fibers containing PSLI in guinea pig bronchi and a nerve net in the hypothalamus.

Chromatography of PSLI from extracts of stomach or trachea by gel filtration gave only one major peak of immunoreactivity which eluted between ^{125}I-physalaemin and ^{125}I-[Tyr^8]-bombesin; i.e., ca. 1700 daltons. Subjecting this pooled material to digestion by extremely high ratios of proteases to substrate (>1000:1) and reanalysis on P-4 columns revealed that its elution profile remained unchanged: PSLI is thus refractory to trypsin and α-chymotrypsin. In a parallel experiment, CNBr digestion gave the same results. PSLI is retained by alkylsilane (C_{18}) columns; it eluted in 35% methanol whereas ^{125}I-physalaemin eluted in 50% methanol. PSLI did not bind to Con-A Sepharose columns.

The proposed course of research rests with the isolation and sequencing of this new neuropeptide from mammalian tissue. Initially, several other means of extraction are now being tried in order to increase the yield of PSLI; this includes use of various organic and inorganic acids, organic solvents and alcohols. Anion and cation exchange resins will be evaluated as a means to eliminate the bulk of extraneous materials. Extraction with organic solvents would eliminate problems of lipid contamination. A partially purified preparation will be absorbed on and eluted from an immunoaffinity column and the salts removed by an alkylsilane resin. Once a highly purified preparation is available, sequencing will be carried out by mass spectrography headed by J. R. Hass. Using new equipment with a fast argon beam - specifically designed for determining the molecular weight of trace quantities of materials - the sequence of 100 ng should be possible. Of course, once the sequence has been verified, then it can be synthesized, antibodies prepared and the spectrum of its biological activity determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance of discovering a unique neuropeptide in mammalian tissue can not be understated: Every new peptide, hormone or factor that can be isolated and characterized adds to our knowledge of the regulation and control of physiological events. Each of these newly uncovered compounds then opens an undreamed of vista for future avenues of research: the unlocking of a Pandora's box of scientific exploration. Control of biological activity appears to involve a multitude of interconnecting facets with considerable redundancy and multiple back-up systems for a finely tuned system with a built-in fail-safe mechanism. However, unless science can fully understand these very basic interactions, it would be difficult to assess the effects of toxic substances on the change in animal physiology and behavior.

PUBLICATIONS

Lazarus, L. H. and DiAugustine, R. P.: Radioimmunoassay for the Tachykinin Peptide Physalaemin: Detection of a Physalaemin-like Substance in Rabbit Stomach. Anal. Biochem. 107: 350-357, 1980.

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Lazarus, L. H., DiAugustine, R. P., Khan, M. N., Jahnke, G. D., and Erisman, M. D.: Application of a Sequence-specific Radioimmunoassay for the Carboxyl-terminal Region of Adrenocortictropin. Clin. Chem. 27:549-552, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25017-01 LPFT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chemical Induction of Respiratory Burst in Macrophages

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

PI:	E. G. Tombropoulos	Research Chemist	LPFT	NIEHS
	R. P. Mason	Research Chemist	LEB	NIEHS
OTHERS:	W. Gibson	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Toxicology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Among the metabolic reactions that are stimulated by phagocytosis is the respiratory burst. During the respiratory burst some highly reactive oxygen species such as $\dot{O}_2^-$, H_2O_2, and OH^- are generated which in combination with lysosomal enzymes are able to kill a large number of microorganisms.

Paraquat (methyl viologen) and nitrofurantoin were examined as to their chemical induction of "respiratory burst" by non phagocytizing cells. These compounds would enter cells, accept electrons from one of the electron carriers and direct a portion of their electron flow to the production of $\dot{O}_2^-$, H_2O_2, and OH^-.

In addition SOD, catalase and mannitol were examined as scavengers of these oxygen reactive species.

PHS-6040
(Rev. 2-81)

METHODS EMPLOYED: Lung lavages from rabbits were obtained using antiseptic techniques. The lavages were centrifuged at 600 g for 10 minutes and the lung macrophages isolated and resuspended in the incubation medium (Medium 199 with Hank's salts 3] mg penicillin and 50 mg sterptomycin). The final cell concentration was 1.5 x 10^6 to 2 x 10^6 cells/ml. Five milliliters of the suspension was incubated in 25 ml culture flasks. Three hours after the initial incubation the majority of the cells were adhered in the flask, the medium was poured off and new medium was added containing the test toxic material.

The following concentrations were used; paraquat .5mM, Nitrofurantoin 0.1 mM, SOD 0.25 mg/ml, catalase 2 mg/ml, mannitol 0.8 mM. During the O2 measurements 3.5 x 10^6 cells were used.

These experiments were designed to answer the following questions: 1. Does paraquat and nitrofurantoin kill alveolar macrophages by the production of active oxygen species? 2. Is the effect of paraquat and nitrofurantoin on alveolar macrophages reversible by SOD which destroys superoxide, by catalase which disproportionates hydrogen peroxide, and by mannitol which traps the hydroxy radical? Does paraquat increase the cyanide resistent respiration of macrophages? 4. Does catalase aleviate the effect of paraquat on the cyanide resistent respiration?

MAJOR FINDINGS AND PROPOSED COURSE: Paraquat (5mM) decreases the viability of alveolar macrophages to 50% as compared to controls. Catalase protects these cells increasing the viability to 68%, whereas SOD increases the viability to 65%. The combination of SOD and catalase raises the cell viability to 77%. Mannitol had no significant protection.

The viability of alveolar macrophages decreases th 50% as compared to controls in the presence of 0.] mM nitroflurantoin. SOD provided partial protection by raising the cell viability to 62%, catalase raises it to 60% and mannitol to 60%.

The difference in concentration between paraquat and nitrofurantoin in producing the same effect may be due to difference in permeability, it is more difficult for paraquat to enter the cells than nitrofurantoin.

The results indicate that both $O_2^{\cdot-}$, and OH^- are important in killing alveolar macrophages. The two enzymes catalase and SOD act to lower the concentration of the chemical reactants and therefore aleviate their effects. The addition of SOD and catalase provide only partial protection from paraquat and nitrofurantoin because the two chemicals enter the cell and the enzymes do not. Therefore the only protection they can provide is from the reactive species of oxygen in solution which presumably attack the surface of the cell.

In a second series of experiments the enhancement of CN^- resistent respiration by paraquat was tested. Fresh alveolar macrophages were suspended in F-12 medium and the O_2 consumption was measured by an oxygen prove. KCN (0.013M) was added and the O_2 consumption was measured again. At paraquat concentrations of; 4.3 mM 8.8 mM, 11 mM, 22 mM, and 33 mM, the following CN^- resistent respiration as per cent of the resting cell respiration (without CN^-) was observed; 4.3%, 28.23%, 28.64%, 34%, and 40%.

The addition of catalase reduced the effect of paraquat (33 mM) by 50%, apparently recovery lost O_2 from hydrogen peroxide. The above results on the viability of alveolar macrophages and respiration agree and both point out to the production of oxygen species deletirious to the cell which can be scanvenged by catalase and SOD. Chronic exposure to low levels of chemicals may initiate low levels of free radical chain propagated reactions which can lead to lipid alteration on the membranes of alveolar cells. This also can be augmented by phagocytosis of microorganisms. Therefore it is importnat to examine the synergistic effect of chemical and biological induced respiratory burst. In addition the comparison of alveolar and peritonial macrophages is important because macrophages derived from the two sources have different metabolism associated with respiration.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Lung macrophages are among the first lung cells coming into contact with inhaled toxic materials. Any, therefore chemical that alters their function in defending the lung or secreting toxic materials which are deleterious to the pulmonary tissue is of highly importance to the program of the Institute.

UNIVERSITY OF CALIFORNIA - DAVIS, CALIFORNIA 95616
(222 Y01-ES-0056)

TITLE: The Biology of Non-ciliated Bronchiolar Cells (Clara Cells) In Vitro

CONTRACTOR'S PROJECT DIRECTOR: Charles Plopper, Ph.D.

PROJECT OFFICER (NIEHS): Karen S. Sonstegard, Senior Staff Fellow, Pulmonary Cell Biology Group, LPFT

DATE CONTRACT INITIATED: July 1, 1980

CURRENT ANNUAL LEVEL: $40,000

PROJECT DESCRIPTION

OBJECTIVES: Non-ciliated Clara cells comprise a majority of the mucosal cell population in peripheral lung bronchioles. Morphologic and histochemical studies suggest Clara cells have a secretory function. The possible importance of Clara cells in toxic reactions of distal airways has come to light with the demonstration of high mixed-function oxidase (MFO) enzyme activity. Chemical toxins including carcinogens require MFO-catalyzed activation to form cytotoxic and/or carcinogenic metabolites. It has been shown that Clara cells respond adversely to hydrocarbons, ozone, nitrogen dioxide, hyperoxic conditions and cigarette smoke and are, therefore, likely to play a key role in the initiation of pulmonary injury and disease. Our objectives are to study the factors and mechanisms that induce and control bronchiolar Clara cell proliferation, differentiation and secretion. To facilitate these studies, we are developing in vitro cell and organ culture systems with which we can examine the biology of bronchiolar epitheliu. Our research plan is, (1) to define the minimal cell and organ culture conditions necessary for maintenance of viable bronchiolar epithelium, (2) to define the specific culture conditions necessary for proliferation and determine the hormones required for differentiation and/or proliferation, and (3) to determine the influence of extracellular matrix components on airway cell growth and differentiation. Baseline steady-state parameters for bronchiolar epithelium will be determined from whole lung and isolated airways.

METHODS EMPLOYED: The first task in developing the in vitro models for Clara cell studies is to obtain quantitative information on the distribution of Clara cells and other cell types in conducting airways. The reative frequency of the major cell types including an assessment of the "non-ciliated" cell category in various sized airways of 10-20 week old, white New Zealand rabbits will be investigated. Cytochemical and ultrastructural characteristics of the non-ciliated cells will be established. The material to be investigated is a) isolated airways from adult rabbit lungs and b) intact adult rabbit lungs. Comparison beween the two types of materials will allow judgement of the adequacy or inadequacy of the isolation procedures used to remove the lung parachuma from the conducting airways. If needed, similar studies will be repeated in other species in order to select an accessible, suitably sized airway with a majority of Clara cells for in vitro study. The cellular composition, viability and ultrastructural integrity of airway explants will be assessed qualitatively by light and transmission microscopy at different times of in vitro cultures. The cellular response to culture conditions in terms of proliferation and differentiation will be

determined morpohologically and quantitatively by autoradiography. The in vitro Clara cell studies to be conducted are: (1) the Clara cell life cycle, the control of Clara cell turnover and differentiation by hormones. (2) The mode and mechanisms of Clara cell secretion following specific stimulation with hormones and drugs and, (3) the response of Clara cells to reactive gases.

MAJOR FINDINGS AND PROPOSED COURSE: Three types of nonciliated secretory epithelial cells are thought to contribute to the mucous lining of intrapulmonary airways: mucus cells, serous cells and Clara cells. The Clara cell is distinguished from the other two by low cuboidal shape, presence of membrane-bound electron-dense ovoid secretory granules, abundant apical agranular endoplasmic reticulum (AER) and its location as the lining cell of distal conducting airways (bronchioles). The present study was designed to define the distribution of the Clara cell within the intrapulmonary airway tree. We have correlated airway size, generation of branching, and airway wall components with Clara cell morphology and abundance in the major (axial) airway and its first intralobar branch of the right apical lung lobe. The percentages of ciliated, nonciliated and basal epithelial cells were quantitated by LM. Basal cells and peribronchial cartilage were absent below the fourth intrapulmonary generation in both the axial airway and its first major branch. Nonciliated cells were more abundant distally, constituting 21% to 45% of the epithelium in cartilaginous airways and 35% to 65% in noncartilaginous ones. In the first 3 airway generations nonciliated cells were without large apical protrusions but were covered by long microvilli. Cells of more distal airways had prominent apical protrusions and lacked microvilli. Nonciliated cells in all airways had abundant apical AER, granular endoplasmic reticulum (GER) and electron-dense ovoid granules. Cells in proximal airways were columnar rather than low cuboidal and had electron-lucent granules and more GER. Only nonciliated cell shape, luminal surface and granule abundance varied with airway size and generation. We concluded that one cell type (the Clara cell) is the nonciliated secretory cell lining all intrapulmonary airways in the rabbit lung. Similar studies are being carried out in rat and hamster lung. Assessment of isolated adult airways and explants is underway.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The structural complexities of whole lung make it difficult to investigate nonrespiratory functions of various pulmonary cells, particularly those which occur in relatively low numbers in the majority of the small airways (e.g., Clara cells). Our specific aim is to develop in vitro model systems starting with whole isolated airways. The importance of studies of this nature lies in the fact that Clara cells are a known target for several environmental factors which may be the cause of pulmonary injury and disease. Yet, our understanding of the function and life cycle of these cells is still very rudimentary.

OAK RIDGE NATIONAL LABORATORIES, Oak Ridge, Tennessee 37830
(222Y01-ES-80041)

TITLE: Study of Antigenic Markers in Developing Epithelial Neoplasia

CONTRACTOR'S PROJECT DIRECTOR: S. J. Kennel, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $103,417

PROJECT DESCRIPTION

OBJECTIVES: This study involves the expression of antigenic markers on tracheal epithelial carcinoma cell lines as they progress from preneoplastic to neoplastic cell populations in tissue culture. Cell lines have been established by in vitro exposure of primary tracheal explants to carcinogens, DMBA and MNNG. Several of these continuous cell lines were not tumorigenic when inoculated into compatible hosts, but became tumorigenic upon passage in vitro. Tumorigenic cell passages were weakly immunogenic in syngeneic animals eliciting both cellular and humoral immune responses. Two major classes of tumor antigens that do not appear on normal (primary) tracheal epithelial cells have been identified using immune rat serum. The complexity of the syngeneic response in defining antigens appearing on malignant phase cell passages has prompted us to produce monoclonal antibodies. Several monoclonal antibodies that react with tracheal carcinoma cells and not normal outgrowth cultures have been identified. We are now investigating the appearance of tumor antigens defined by both monoclonal antibodies and immune sera as preneoplastic cultures progress from non-tumorigenic to tumorigenic populations in vitro.

METHODS EMPLOYED: Induction of transplantation immunity or repeated immunizations with lethally x-irradiated cells (10,000 R) are being used as a source of immune sera and as spleen cell donors for fusions with mouse P3-X63-Ag8 myeloma cells for hybridoma antibody production. Serological recognition of cell surface antigens using hybridoma or serum antibodies 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 (Braslawsky et al., 1981) is of the first type. This test can quantitate the amount of tumor antigen on neoplastic cell populations and determine when these antigenic moieties appear on pre-malignant populations. The fluorescent antibody test combined with flow-cytometry is of the second type, which analyzes cell populations for fluorescent yield per cell as well as enumerates the percentage of antigen positive cells.

MAJOR FINDINGS AND PROPOSED COURSE: Initial studies on the ability of two malignant phase tracheal cell lines to induce transplantation-resistance in syngeneic F-344 rats have been completed. Both humoral and cell-mediated immune responses were demonstrated. Sera from transplantation-

resistant rats were used to identify antigens associated with the transformed phenotype. Such antigens were not detected on non-transformed epithelial cell lines. However, absorption experiments showed two antigenic groupings: One that was expressed on several tumors of the F-344 rat (cross reactive) and one limited to the immunizing cell line (tumor) specific). Immunizations with lethally irradiated cells were also effective. Antibody titers similar to those obtained in sera of rats having transplantation-resistance were obtained using lethally irradiated (10,000 R x-irradiation) 2-10-1 cells as immunogen.

Syngeneic immune serum recognizing cell surface antigens has little reactivity for non-transformed tracheal cells. However, the range of antibody specificities is complex, including at least two classes of antigens on 2-10-1 cells. The development of hybrid cell cultures that produce monoclonal antibody allows for better identification and quantitation of individual cell surface antigens, as well as production of unlimited quantities of monospecific antibody. Spleen cells from several rats have been sufed to the mouse myeloma, P3-X63-Ag8 using polyetholene glycol. About 1% of all hybridoma clones produced with rat spleen cells from syngeneic immunizations produce antibody to 2-10-1 cells. To date, four hybrid lines have been recloned and monoclonal antibody concentrated from supernatant fluids by 50% saturated ammonium-sulfate precipitation. Three hybrid clones were reactive with 2-10-1 cells, and showed no reactivity for normal tracheal epithelial cells. These monoclonal antibodies are currently being characterized for binding activity against other transformed cell lines as well as normal tissues or other tumors from the Fischer rat.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The appearance of tumor markers which can be recognized immunologically during the period when cells have altered (transformed) phenotypes, but lack oncogenic ability (malignant neoplasia), would be invaluable in understanding the oncogenic process. These studies will provide information on the appearance of tumor antigens after carcinogenic insult and the frequency of antigen positive cells in exposed populations before the appearance of neoplastic cell populations. The isolation of antigen-positive phenotypes in mixed populations will also allow study of progenitor-progeny relationships that have evolved as a result of carcinogenesis initiation. This will enable us to determine if antigen positive cells have a selective growth advantage over non-antigenic bearing cells and whether the quantity of antigen expressed changes as a function of neoplastic differentiation in vitro. Assay systems used in these experimental animal model systems in the future will provide the basis for defining human respiratory tract tumor markers and may also be useful for short term testing of malignant transformation potential of putative carcinogens.

PUBLICATION

Braslawsky, G. R., Steele, v., Kennel, S. J., and Nettesheim, P. Syngeneic immune response to rat tracheal epithelial cells transformed in vitro by N-methyl-N'-nitro-N-nitrosoguanidine. Br. J. Cancer, in press.

UNITED STATES DEPARTMENT OF ENERGY - OAK RIDGE OPERATIONS
Oak Ridge, Tennessee 37830
(222Y01-ES-80032)

TITLE: Susceptibility of Preneoplastic Epithelial Lesions to Toxic and Carcinogenic Insults

CONTRACTOR'S PROJECT DIRECTOR: A. J. P. Klein-Szanto, M.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

PERIOD COVERED: October 1, 1980 - September 30, 1981

CURRENT ANNUAL LEVEL: $165,000

PROJECT DESCRIPTION

OBJECTIVES: Our research work was directed toward:

1) The characterization of different dark cell subpopulations in preneoplastic lesions of the respiratory tract epithelium using mrophology, histochemistry and cell kinetics.

2) Investigating the behavior of cell culture derived epithelia repopulating denuded rat tracheas and the effect of reexposing them with very low doses of chemical carcinogens.

3) Establishing of a two stage model in the tracheal transplant model.

4) Developing and studying the effect of slow release systems for particulate materials in the tracheal transplant model.

METHODS EMPLOYED: Dark cell epithelial cells were studied in preneoplastic lesions of heterotopic syngeneic tracheal transplants treated with DMBA (200 μg - 4 weeks), and in denuded tracheas repopulated with an epithelium derived from a cell line originally treated with the same carcinogen and presenting tumorigenic characteristics. The animals carrying these tracheal transplants were injected with ^{3}H Tdr one hour before sacrifice. One hour pulse labeling as well as chasing of the label during the first 72 hours after ^{3}H Tdr inoculation were carried out. All tissues were embedded in Epon and autoradiograms were produced using one micron thick sections. The repopulation studies were carried out in 3 cell lines (2 preneoplastic originally treated with DMBA, and on non-treated "normal" cell line). Four weeks after inoculation of the cell lines into the denuded tracheas, the fully reconstituted epithelai were exposed to 0.05 μg of MNNG. The two stage carcinogenesis experiments were carried out by expoisng rat tracheal transplants to subthreshold doses of carcinogen (35 μg DMBA) followed by TPA as promoter (100 μg in cholesterol pellets). The lesions were analyzed at 2 weeks, 3, 10, and 12 months. The in vivo release of nickel subsulfide was studied, using either stearyl alcohol or polyglycolic acid matrices. The effects on the tracheal epithelium were studied using paraffin sections.

MAJOR FINDINGS AND PROPOSED COURSE: Dark basal cells were observed in preneoplastic epithelia of DMBA treated tracheal transplants. A small percentage of these cells exhibited involutional characteristics, whereas the vast majority showed either normal or hyperactive features. In order to determine how many dark cells participate in the proliferative pool, a label chasing experiment was performed by couting the number of grains 1 to 72 hours after inoculation of ^{3}H Tdr. A cell line repopulated trachea was selected because of the uniformity of the pattern and distribution of dark cells in the reconstituted epithelium. Using the cell line 1000 M, a fully reconsituted epithelium containing 15-20% dark cells in the basal layer is readily available 2 weeks after inoculating the cells into the denuded tracheas. This experiment showed that only 3-4% of the total dark cell population does not divide at 72 hours (involutional or slow cycling cells) whereas the large majority of dark cells to divide between 24 and 48 hours. Other experiments are now underway to determine if these cells are capable of dividing more than once, and study the characteristics of the offspring cells.

Two cell line-derived epithelia originally treated with DMBA (D55 and 4081) and one spontaneous "normal cell" line were exposed to MNNG. No lesions (except atrophic epithelia) were seen in the normal cell line at 3 and 5 months after carcinogen inoculation. On the other hand, the two preneoplastic cell line-originated epithelia gave rise to many squamous carcinomas as early as 3 months after inoculation. The epithelia not treated with carcinogen gave rise to some malignant tumors later than 5 months after sham treatment with solvent. This experiment is still being pursued and similar experiments, using these and other cell line originated epithelia treated with the promoter TPA, are being planned for the near future. The two stage carcinogenesis experiment using DMBA as initiator and TPA as promoter have been continued using cholesterol as matrix for these agents. The lesions and alterations of the *in vitro* growth potential of epithelial cells produced by cholesterol seem to be banal and have demonstrated to be reversible. The animals treated with cholesterol blanks either as initiator or promoter did not show preneoplastic or neoplastic lesions. The two stage experiment per se has shown that with a very low DMBA dose, the tumor incidence was 1 in 48 tracheal transplants, whereas when this DMBA initiator is followed by TPA promotion, the incidence (at 14 months) was 5/48. The experiment will be continued up to the 24th month, and more tumors are likely to arise in the two stage carcinogenesis group. In addition, we will attempt to study the effect of the dose rate of the initiator and the time interval between initiation and promotion on the type of lesions produced during the carcinogenesis process as well as on the final tumor incidence.

The possibility of using particulate material implicated in human carcinogenesis in our tracheal transplant model has been hampered by the lack of an adequate release system permitting a slow and homogeneous release of poorly soluble particles. We have recently studied the *in vivo* release rates of nickel subsulfide in polyglycolic acid and stearyl alcohol. This last substance mixed with beeswax (ratio 9:1) has proved to be an adequate vehicle for these particles, permitting a sustained release for more than 16 weeks. Similar results are now being expected with arsenic trioxide. These substances as well as asbestos and glass fibers will be using in future one stage and two stage carcinogenesis studies using heterotopic rat tracheal transplants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The studies represent attempts to better understand the multifactoral nature of carcinogenesis, especially the influence of the combined or sequential effects of two or more chemical carcinogens and/or promoter in the respiratory tract. In addition, some new insight into novel morphological, cell kinetics and histochemical characteristics of preneoplastic lesions will help to better type, classify and eventually diagnose these putative cancer precursors. These types of experimental data are essential to design, in the future, rational approaches for diagnosis and intervention therapy in groups of individuals at high risk to develop lung cancer.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY
Summary Statement

Because the detection of environmental agents which affect reproduction or produce birth defects is unsure and the underlying biological mechanisms which account for these major health problems are unclear, the Laboratory of Reproductive and Developmental Toxicology seeks to bridge the gap between the most molecular aspects of reproductive biology and endocrinology and the more applied problems associated with the detection of hazardous chemicals, the extrapolation of laboratory data to man, and the estimation of human risk.

The Laboratory directs its research efforts to three major areas at the present time: (1) male and female reproductive processes; (2) teratogenesis; and (3) postnatal effects of gestational chemical exposure and is organized into three research program areas: Experimental Teratogenesis, Reproductive Toxicology, and Transplacental Toxicology. Extensive collaboration exists between these groups; thus, the current research projects for the Laboratory are listed below as integrated programs at various levels.

I. MOLECULAR LEVEL

A. Hormone-Related Gene Action in the Male and Female Genital Tract

- o Hormone regulation of prostate and seminal vesicle protein synthesis
- o Molecular characterization of androgen dependent rat seminal vesicle genes
- o Molecular cloning for androgen dependent rat prostate genes
- o Molecular cloning of human prostate genes derived from benign hyperplastic tissue

B. Biochemical Basis of Estrogen Action

- o Characterization of nuclear events involved in estrogen action in the mouse uterus
- o Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites
- o Analysis of proteins of the mouse uterus which are involved in the estrogenic response

C. Biochemical Basis for Craniofacial Teratogenesis

- o Glucocorticoid involvement in prenatal craniofacial development

- o Role of epidermal growth factor (EGF) in prenatal growth and differentiation

- o Biochemical basis of TCDD teratogenesis

II. ULTRASTRUCTURAL/CELLULAR LEVEL

A. Ultrastructural Changes as Predictors of Functional Abnormalities

- o Correlation of scanning and transmission electron microscopy observations with biochemical, histological, or functional changes in the male and female mouse genital tract

B. Toxication/Detoxication of Environmental Chemicals by Target Tissues Related to Reproduction

- o Polycyclic hydrocarbon metabolism by rodent testes

- o Development of a coupled system of microsomal enzymes and cultured rodent embryos to determine the role of metabolism in teratogenicity

- o Characterization of diethylstilbestrol metabolism and the elucidation of metabolic pathways which produce metabolites of differing biological activities

C. Toxicology of Early Development

- o Interspecies *in vitro* fertilization as an indicator of reproductive capacity

- o *In vivo* development of *in vitro* fertilized eggs to assess potential of developmental defects

III. TISSUE/ORGAN LEVEL

A. Cultured Embryos

- o Establishment and biochemical/physiological/morphological characterization of an *in vitro* system to grow and maintain whole rodent fetuses during critical periods of organogenesis

- o Studies of the Fetal Alcohol Syndrome

B. Isolated Development of Fetal Organs

- o Establishment and biochemical/physiological/morphological characterization of an *in vitro* system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity

- o Determination of oxidative metabolism of estrogenic chemicals in organ cultures of fetal tissues.

- o Morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice

- o Morphological characterization of long-term fetal tissue grafts

- o A model system to assess toxic effects on gametes, early development, pre- and post-implantation embryos, and fetuses

C. Cell Cultures as Model Systems

- o In vitro neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally related chemicals

IV. WHOLE ANIMAL STUDIES

A. Toxicology

- o The teratogenicity of anticonvulsant agents and structurally related chemicals

- o The teratogenicity of steroids and steroidal alkaloids

- o Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed in utero to diethylstilbestrol

- o Effects of DBCP on male reproductive function in mice

- o Effects of kepone on male reproductive function in mice

B. Data Extrapolation to Man and Risk Estimation

- o Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans

- o Quantification of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk

- o Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons

- o Studies of human facial clefting

Summaries of these projects are presented below: details of the work appear in the individual annual report.

I. MOLECULAR STUDIES

A. Hormone-Related Gene Action in the Male and Female Genital Tract

Hormone regulation of prostate and seminal vesicle protein synthesis: Protein synthesis patterns analyzed by two dimensional (2D) gel electrophoresis in the prostate and seminal vesicle of castrated and testosterone stimulated rats indicate that a major group of secretory proteins in both organs is under androgen control. Both organs have a high concentration of poly(A^+)-mRNA which code in a wheat germ translation system for major polypeptides. Two major poly(A^+)-RNA's from prostate (labeled β and δ) code for the subunits of the major secretory product referred to as prostate binding protein or prostatein. These two prostate poly(A^+)-mRNA make up 30-40% of the total poly(A^+) of the prostate. A third major prostate poly(A^+)-mRNA (α) codes for a larger (22,000 dalton) secretory protein. Likewise, rat seminal vesicles have two major poly(A^+)-mRNA's (40%) which code for two major seminal vesicle proteins which are androgen responsive (IV and V).

Molecular characterization of androgen dependent rat seminal vesicle genes: Double-stranded complementary DNA for two major seminal vesicle poly(A^+)-mRNA's was prepared (ds cDNA to mRNAsv). The two seminal vesicle synthetic genes were purified and isolated and restriction maps generated. It appeared as if the structural parts of these two seminal vesicle genes have considerable sequence homology. 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). Presently, 13 signals from three million clones of the λ charon 4A rat library have been isolated. These phage clones, containing an average of 15 kb of rat DNA, should be quite useful for mapping the chromosomal genes for SVS protein IV and V. The seminal vesicle ds cDNA to mRNAsv IV and mRNAsv V has been cloned and probes developed for these structural genes.

Molecular cloning of androgen dependent rat prostate genes: Double-stranded complementary DNA (ds cDNA 10-13s) to androgen dependent α, β, and δ poly(A^+)-RNA from ventral prostate was prepared using reverse transcriptase. The ds cDNA was treated with S_1 nuclease, tailed with terminal transferase and cloned in PBR322. Clones were identified by hybrid arrest translation of prostate mRNA which had been hybridized with insert DNA derived from candidate clones. Thus far three clones have been identified which carry the structural gene information for α, β, and δ polypeptides. Identification of each of these clones has been verified by hybridization of probes nick-translated from the

respective inserts to ventral, lateral and dorsal prostate poly(A^+)-mRNA northern transferred to nitrocellulose filters. In the case of lateral and dorsal mRNA, no corresponding bands were found implying that these lobes of the rat prostate are biochemically different from the ventral prostate. Detailed restriction maps for each insert have been developed.

Molecular cloning of human prostate genes derived from benign hyperplastic tissue: As a source of human prostate tissue, we have used surgical specimens frozen in dry-ice immediately following excision of hyperplastic prostate tissue. After accumulation of 30 gm of tissue, poly(A^+)-mRNA was isolated and ds cDNA prepared by reverse transcriptase and E. Coli polymerase I in a two step reaction. After S_1 nuclease treatment and tailing with terminal transferase, the ds cDNA was cloned in PBR322 and 150 transformants found to be ampicillin sensitive and tetracycline resistant. Screening of this human prostate structural gene library is currently being carried out to identify clones with large inserts. We are also in the process of obtaining prostate tissue from accident victims with which to establish a gene library for the normal human prostate.

B. Biochemical Basis of Estrogen Action

Characterization of nuclear events involved in estrogen action in the mouse uterus: The second translocation of hormone receptor complex to the nucleus after exposure to estrogen, which occurs in the mouse uterus, suggests two events in estrogen receptor action. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear event. The role of this event in estrogen action in the mouse reproductive tract, with particular regard to the actions of hormonally active environmental chemicals, is being studied. Receptor synthesis, RNA polymerase activities, DNA polymerase activities and glucose oxidation/utilization are also being investigated. Progesterone inhibition of uterine growth is also being studied to determine the biochemical events involved in estrogen growth promotion. Recent studies using steroid autoradiography raise the possibility that the second nuclear accumulation of estradiol-receptor complex may be related to its redistribution to different cell types within the tissue. Thus, estrogen action in the mouse uterus may involve sequential stimulation of various tissue compartments for its expression.

Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites: In order to determine whether the metabolism of DES results in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an *in vivo* bioassay and an *in vitro* receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity, while certain 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 indanyl-DES and pseudo DES which are DES analogs as well as possible metabolites showing receptor binding comparable to DES, but with 20-100 times less biological activity. These studies have suggested a mechanism of differential genomic stimulation and altered clearance of analog receptor complexes from the target cell nucleus.

Analysis of proteins of the mouse uterus which are involved in the estrogenic response: Intracellular uterine proteins were labeled with [^{35}S] methionine using an in vivo stimulation/in vitro labeling technique. Two-dimensional gel electrophoresis was employed to detect any qualitative protein changes due to estrogen administration. Uterine tissue was subfractionated in order to determine what protein changes may have occurred in the individual cell fractions. Estrogen treatment resulted in the appearance of two uterine proteins in the 0.4 M KCl nuclear extract and a significant decrease in several proteins in the cytoplasmic fraction. Studies are in progress to identify and determine a functional role for these proteins as well as to identify uterine secretory proteins as markers of uterine cell function and for their possible physiologic roles.

C. Biochemical Basis for Craniofacial Teratogenesis

Glucocorticoid involvement in prenatal craniofacial development: Depending on strain, glucocorticoids are potent inducers of cleft palate in experimental animals. The biochemical basis for this strain susceptibility appears to be due, in large part, to elevated levels of glucocorticoid receptors in the craniofacial tissues of fetal mice. Mesenchymal cells have been established in culture from the responsive A/J and nonresponsive C57 mouse palatal shelves. These cells are being examined in cell culture using a variety of parameters, including cell surface glycoproteins, to further define the biochemical basis for glucocorticoid induced teratogenicity.

Role of epidermal growth factor (EGF) in prenatal growth and differentiation: EGF is a potent polypeptide capable of stimulating proliferation and differentiation in a number of cell types in vitro and in vivo. Our recent studies have provided evidence for a fetal form of EGF that appears at midgestation and is presumably important for the development of a number of tissues including the secondary palate. Studies are in progress to characterize more extensively the biochemical properties of fetal EGF and compare it to the properties of adult EGF. EGF and glucocorticoids are known to act synergistically and therefore studies are in progress with both EGF and glucocorticoids in palate cell culture.

Biochemical basis or TCDD teratogenesis: Although, TCDD is the most potent teratogen known for experimental animals, it induces relatively few types of congenital anomalies including cleft palate. Strain differences in response to TCDD-induced cleft palate correlate well with elevated levels of TCDD cytoplasmic receptors in craniofacial tissues from sensitive fetal mice. Mesenchymal cell lines from palatal shelves have been established from the sensitive C57 and nonsensitive AKR strains to further explore the nature of TCDD-induced biochemical effects which could account for its teratogenesis.

II. ULTRASTRUCTURAL/CELLULAR STUDIES

A. Ultrastructural Changes as Predictors of Functional Abnormalities

Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histological or functional changes in the male and female mouse genital tract: Studies have demonstrated that scanning

electron microscopy (SEM) provides a tool for the detection of early neoplastic changes. The surface ultrastructural features of the lumen of the female mouse genital tract was evaluated during the estrous cycle and during development in normal CD-1 mice. The hormone dependence of fine structural features of the cell surface was demonstrated in ovariectomized, hormone-treated females where various characteristics of intact animals were experimentally induced. Subsequent studies on DES-treated mice indicate that cell surface features are directly related to abnormal cell differentiation. Changes in the cell surface are correlated with alterations in the histological features of DES-exposed offspring. Transmission electron microscopic studies have shown that the most striking and reproducible ultrastructural lesion in the uteri of prenatally-DES treated females is an abnormal stromal-epithelial interface.

B. Toxication/Detoxication of Environmental Chemicals by Target Tissues Related to Reproduction

Polycyclic hydrocarbon metabolism by rodent testes: Polycyclic hydrocarbon activating and deactivating enzyme systems have been studied in the rodent testes. Benzo(a)pyrene aryl hydrocarbon hydroxylase (AHH), epoxide hydrase (EH), glutathione S-transferase (GSH-ST), and cytochrome P-450 were determined in both the seminiferous tubule and interstitial cellular compartments. Similar studies were done with prostatic tissue. Tetrachlorodibenzo-p-dioxin (TCDD) induced AHH and EH in both the testis and prostate gland; a surprising 150 fold increase in AHH was noted in the prostate gland. AHH, EH, and P-450 were each increased in hypophysectomized male rats following treatment with luteinizing hormone (LH) while follicle stimulating hormone (FSH) or testosterone had no effect. These results suggest that LH-associated induction of testicular AHH, EH, and P-450 content is associated with the interstitial cell compartment. Since toxic effects of arene oxides on male germ cells are affected by various pharmacokinetic factors not represented in cell-free systems, the isolated perfused testis (IPT) was utilized to better understand the testicular metabolism of chemicals. The isolated testis was perfused with radio-labelled benzo(a)pyrene and metabolites determined using high pressure liquid chromatography. Metabolites in the perfusate differed from those detected in testicular tissue, and testicular metabolism contrasted with that noted previously for the liver. The role of testicular activation of chemicals is important to the understanding of reproductive and genetic effects of chemicals on male germ cells.

Development of a coupled system of microsomal enzymes and cultured rodent embryos to determine the role of metabolism in teratogenicity: Many toxic chemicals and most mutagens and carcinogens require metabolic activation of the substrate to an active form. Microsomal biotransformation enzymes have recently been coupled to embryos grown in culture in a manner analogous to the Ames test where an S-9 activating system is in contact with cultured Salmonella bacterial strains. It has been demonstrated that cyclophosphamide, an anticancer agent which requires metabolic activation, has no adverse developmental effects on cultured embryos unless the microsomal enzymes are present. Enzyme and cofactor requirements are being optimized for embryonic growth and enzymatic activity. Thus, a mammalian test system has been

developed which might quickly predict environmental chemicals which are either direct or indirect acting teratogens.

Characterization of diethylstilbestrol metabolism and the elucidation of metabolic pathways which produce metabolites of differing biological activities: It has been demonstrated that peroxidase, an enzyme inducible in estrogen target tissues, is able to metabolize DES to its major metabolite, β-dienestrol. Bioactivation of DES was determined by the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from a target tissue, the mouse uterus. This peroxidase activating system was also studied in the hamster kidney, a non-genital target tissue for DES. The peroxidatic activity of prostaglandin synthetase was found to catalyze the oxidative metabolism of DES in vitro and in cell culture. The estrogenic activities of a series of DES metabolites and analogs were determined. Results of studies suggest that DES metabolism follows alternative pathways resulting in either metabolites which retain estrogenicity, lack activity or are of ambiguous activity. The latter class includes the indenestrol isomers and pseudo DES. These compounds have a comparable binding affinity to DES but are some 20-150 times less biologically active. Studies on the levels at which they fail to elicit a biological response include estrogen receptor translocation/clearance, DNA synthesis and mitosis. Determination of the biological significance of potentially activated metabolites of DES should aid in generalizations to other classes of estrogenic environmental chemicals.

C. Toxicology of Early Development

Interspecies in vitro fertilization as an indicator of reproductive capacity: Heterologous (human sperm/hamster egg) in vitro fertilization was used to assess fertilizing capacity of human males. These studies identified certain individuals as subfertile who have routinely determined normal sperm number, motility, and morphology. This approach demonstrated that the usual parameters for semen analysis are unsure predictors of male subfertility; only the most extreme semen abnormalities reliably predict human subfertility. Pregnancies occurring during the course of these studies have all been fathered by men with positive in vitro test results. Zona-free hamster eggs have been stored in liquid nitrogen without loss of fertilizing capacity. Improvements in the cryopreservation of sperm samples are also being sought. These preservation procedures will facilitate testing and allow subjects distant from the laboratory to be studied. Thus, the in vitro penetration of laboratory animal ova by human sperm appears to be an improved technique to assess human fertilization potential. The epidemiological application of this noninvasive, fast, and relatively inexpensive technique is being explored.

In vivo development of in vitro fertilized eggs to assess potential of developmental defects: Available methodologies and the public interest in "test-tube" babies stimulated the linking of our in vitro fertilization and embryo culturing techniques. A "test-tube" baby is actually a product of in vitro fertilization, embryo culturing, and transfer to a female recipient. Using the mouse model, it was demonstrated that these in vitro procedures were not associated with morphological abnormalities of the offspring.

III. TISSUE/ORGAN STUDIES

A. Cultured Embryos

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain whole rodent fetuses during critical periods of organogenesis: To aid in the laboratory assessment of teratogens and in the understanding of the molecular mechanisms underlying teratogenesis, an in vitro culture system for rodent embryos has been established. Rat conceptuses of pregnancy day 10 can be grown continuously for 96 hrs with extensive differentiation of major organs. Utilizing this embryo culture, nutritional and hormonal requirements for embryonic development are being studied, and the system's predictiveness for chemical teratogens is being tested. Extensive development of major organs occurs which is comparable to in vivo differentiation during the same period; organogenesis is highly sensitive to direct acting alkylating agents such as tetraethylene melamine (TEM). A microsomal enzyme activation system has been coupled to the embryo culture system which allows the detection of indirect acting teratogens such as cyclophosphamide.

Studies of the Fetal Alcohol Syndrome: The Fetal Alcohol Syndrome is a serious and frequent human birth defect. Our studies using whole embryo culture suggest that ethanol has a direct effect on the developing fetus. Investigations are in progress to more precisely define whether ethanol inhibits cellular proliferation and/or differentiation and to what extent its effects are restricted to the craniofacial region. Future studies will utilize scanning and transmission electron microscopy to more fully characterize the morphological effects of ethanol. In addition, immunofluorescent localization of various macromolecules, such as fibronectin, will be performed to assess the effect of ethanol.

B. Isolated Development of Fetal Organs

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity: The morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice has been established. Explants of fetal mouse gonads and genital tracts maintained in organ culture have been used to determine protein maps of developing tissues derived from DES exposed and unexposed animals. The biochemical studies are correlated with hormone response studies and morphological observations. Recent studies have focused on growth factor requirements for proliferative response to estrogens in vitro. The oxidative metabolism of DES in the fetal genital tract organ culture system was determined; differences in metabolic patterns could be demonstrated in different fetal target tissues.

Morphological and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice: A heterologous organ culture system including DES exposed or unexposed testes or Müllerian ducts has been used to determine the mechanism of Müllerian duct persistence and hyperplasia in DES treated males. Results suggest that the primary site of action is on the duct system rather than

through a failure of the testis to synthesize or release Mullerian inhibiting Hormone.

Morphological characterization of long-term fetal tissue grafts: To overcome difficulties in long-term (greater than two months) maintenance of fetal tissues in vitro, cultured fetal gonads or genital tracts are carried as grafts in appropriately manipulated hosts. Under these conditions, fetal ovaries develop into functional gonads when grafted under kidney capsules. Some of the epithelial abnormalities observed in vivo can be seen in long-term explants of fetal vaginal tissues. Thus, the contribution of the postnatal environment to expression of prenatally induced abnormalities can be studied.

A model system to assess toxic effects on gametes, early development, pre- and post-implantation embryos, and fetuses: Male and female gametes can be exposed to environmental agents either in vitro or in vivo and then be used for in vitro fertilization. Conceptuses can also be recovered following mating of treated animals. The zygote is subsequently cultured and the early embryo (blastocyst stage) transferred to a pseudopregnant recipient. Using this approach, the following parameters can be monitored: sperm motility; in vitro fertilizing capacity; 4- and 8-cell stage formation; morula and blastocyst development; implantation success (resorbed/dead/live fetuses); pregnancy rate; and malformations. Thus, chemical effects on the sperm and ova, early development, preimplantation and postimplantation embryos, and birth defects can be studied. Cadmium chloride had no effect on fertilization or cleavage rates; however, the conceptuses developing to morula and blastocysts was decreased. There was also a significant increase in preimplantation loss in the cadmium-treated group following transfer of embryos. Of those embryos which implantated, the percent of normal term offspring was unchanged by cadmium exposure.

C. Cell Cultures as Model Systems

In vitro neoplastic transformation of embryonic cells by diethylstilbestrol (DES) and structurally related chemicals: DES was shown to morphologically transform cells in culture at doses comparable to benzo(a)pyrene. The cells were tumorigenic when injected into appropriate hosts. Transformation was accomplished in the absence of measurable somatic mutation and of stimulated cell proliferation. Chemicals with structures capable of bioactivation by the peroxidase pathway were efficient transformants. Metabolism of DES to β dienestrol via this peroxidase pathway was shown to occur in this cell culture system.

IV. WHOLE ANIMAL STUDIES

A. Toxicology

The teratogenicity of anticonvulsant agents and structurally related chemicals: The teratogenicity of structurally related (cyclic imides) antiepileptic drugs was studied. Trimethadione, dimethadione, and paramethadione are derivatives of oxazolidinedione; diphenylhydantoin, ethootoin, and mesantoin are hydantoins; and phensuximide, methsuximide, and ethosuximide belong to the succinimide class of compounds. All the

drugs and each of the basic chemicals produced embryotoxic and teratogenic effects. The malformation profile observed in the CD-1 mouse was similar to those described in humans following in utero exposure during anticonvulsant therapy. Comparative computer analysis of the dose-related increase in the incidence of malformations and adult lethality for each of the compounds indicated that the relative teratogenicity of the oxazolidinedione class of anticonvulsants was significantly greater than that of hydantoins and succinimides. A common mechanism of action associated with the imide structure is suggested by the fact that each of these drugs shares a common moiety and all are embryotoxic and teratogenic.

The teratogenicity of steroids and steroidal alkaloids: Glucocorticoids are potent inducers of isolated cleft palate in various mouse strains. The mechanisms appear to be receptor dependent and involve a preferential inhibition of craniofacial growth, which results in delayed palatal growth and failure of palatal fusion. Recent studies have shown that vitamin B_6 can substantially reduce the incidence of glucocorticoid-induced cleft palate. It appears that vitamin B_6 inhibits glucocorticoid binding to cytoplasmic receptors. The steroidal plant alkaloid, jervine, is known to be teratogenic in animals that feed on certain plants, such as veratrum californicum. Jervine is also teratogenic in certain strains of mice. Using radiolabeled jervine, we plan to explore its distribution and basic biochemical effects which account for its teratogenicity. In addition, labeled jervine will be used to investigate the possibility that its teratogenic action is dependent upon binding to the estrogen receptor.

Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed in utero to diethylstilbestrol: Prenatal exposure to DES in mice results in a dose-related decrease in fertility and genital tract abnormalities in the offspring of both sexes; in females, tumors of the vagina, cervix, uterus, and ovary were found. The stage of differentiation of the genital tract at the time of DES treatment was critical to the later expression of reproductive tract lesions. Induction of a benign vaginal lesion, adenosis, occurred later in development than the induction of the malignant vaginal lesion, adenocarcinoma. Immune function in both sexes was altered although not uniformly for males and females. These studies should be pertinent to the development of an animal model for similar human exposures.

Effects of DBCP on male reproductive function in mice: Dibromochloropropane (DBCP) has been shown to be mutagenic and carcinogenic in bacteria and experimental animals. DBCP has been extensively used in agriculture and has been linked to an increased incidence of male sterility among workers in processing plants. Sterility was associated with low sperm counts and a concomitant elevation of plasma gonadotrophins as well as incidence of testicular germinal aplasia from which recovery is uncertain. DNA repair in premeiotic spermatogenic cells of CD-1 mice was induced by a single i.p. administration of DBCP; in contrast, no unscheduled DNA synthesis was induced in spermatozoa irrespective of dose. The exact mechanism of the action of DBCP with respect to germ cell damage is unknown. However, we speculate that dehydrobromination of DBCP might result in a reactive aliphatic epoxide which could then react with cellular macromolecules, especially germ cell DNA.

Effects of kepone on male reproductive function in mice: Kepone, a widely used pesticide, has a significant effect on testicular, epididymal and body weights of male rats. Sperm production was eliminated at the high dose (100 ppm). At 30 ppm, only body weight and cauda epididymal weights were significantly reduced. No recovery of any parameter was observed at the end of a three week post-treatment period. These data indicate an effect of Kepone on male reproductive organ weights and on sperm production. It would be useful to examine human males exposed to Kepone to assess sperm fertilizing capacity using the heterologous *in vitro* fertilization test system.

B. Data Extrapolation to Man and Risk Estimation

Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans: Many of the genital tract lesions observed in mice exposed prenatally to DES have been observed in comparably exposed humans. For example, in the male, epididymal cysts, prostatic inflammation, sperm abnormalities, and cryptorchidism have been observed in both species; in females, vaginal adenocarcinoma has been seen in the prenatally-exposed mouse and human. Good examples of the utility of such studies is the report of retained testes in male mice derived from DES-treated mothers two years before a similar observation was reported in man and the report of dose-related subfertility in female mice two years before comparable reports in woman.

Quantification of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk: When percent malformations are expressed as a function of percent of the LD-50 dose, a family of curves are produced which distinguishes quickly those chemicals which are teratogenic at doses well below maternal toxicity. Thus, a more reliable reference point than dose is established. The relationhship between percent malformations and observable toxicity, such as CNS depression, is also being defined. Analyzing data concerning the teratogenicity of three anticonvulsant agents (trimethadione, diphenylhydantoin, and ethosuximide), it is readily apparent that the greatest clinical risk is associated with the use of trimethadione; the least with ethosuximide.

Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons: In the male gonads, factors which modify toxicity include the pharmacokinetic parameters governing the absorption, distribution, activation, and detoxication of toxicants; covalent binding to macromolecules; and DNA damage as well as DNA repair of damaged germ cells. All of these factors are being studied in our laboratory at the present time. The male germ cells are protected by a biological barrier comparable to that which retards the penetration of chemicals to the brain; permeability constants for the two are nearly identical. Toxication and detoxication processes are present in both the seminiferous tubule and interstitial cellular compartments. The balance of toxication/detoxication processes apparently favors the germ cells; detoxication reactions are relatively more abundant in the seminiferous tubules. Unscheduled DNA repair has been demonstrated in spermatogonia and spermatocytes; spermatids and sperm lack DNA repair capability. The DNA repair capacity associated with spermiogenic cells appears to be dose-dependent and saturatable. Understanding the

pharmacokinetic characteristics of the blood-testis barrier, toxication and detoxication mechanisms as well as DNA repair systems in male gonads will allow a better understanding of species comparison, of reproductive and genetic toxicity, and will increase the reliability of extrapolating laboratory animal data to man and estimating human risk.

Studies of human facial clefting: Cell lines have been established from the dermal fibroblasts from a number of normal and abnormal individuals with facial clefting ranging from 3 months to 37 years of age. Preliminary studies suggest alterations in the level of glucocorticoid receptors in these cells which presumably could indicate a defect which might have adversely affected fetal development. Studies are in progress to determine the level of receptors for other hormones and growth factors and to examine these affected cells for sensitivity to various known or suspected human teratogens. This information may eventually prove useful in genetic screening and counseling for individuals predisposed to genetic and/or environmentally-caused birth defects.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international levels. Laboratory scientists have acknowledged the expertise and resources concentrated in nearby universities. Moreover, we have not hesitated to establish the collaborative efforts with scientists throughout the world necessary to develop quality intramural programs.

Dr. Carter is involved in a collaborative project with Dr. Martin Resnick, Department of Urology, Bowman-Gray School of Medicine and Dr. David Paulson, Department of Urology, Duke University. The research with Dr. Resnick focuses on the identification of seminal plasma proteins with respect to origin. Dr. Paulson provides human prostate tissues which are used to isolate poly(A^+) mRNA from which a cDNA library has been established.

Dr. Hall continues his collaborative studies with the University of North Carolina to investigate effects of therapeutic agents (such as chemotherapy, radiation therapy, and treatment of infertility) on human male reproduction.

Dr. Lee continues his involvement in several collaborative projects. He is collaborating with Dr. Harry Tyrer at the Cancer Research Center, University of Missouri, on a project using lasers to quantitate fluorescent metabolites in a single cell. Studies are also being continued with Dr. Larry Ewing, Johns Hopkins University, concerning Leydig cell function of F_1 males following prenatal exposure to TCDD. A collaborative project with Dr. B. F. Speilvogel, Gross Chemical Laboratory, Duke University, to better understand mechanisms of boron-induced male infertility, organoboron, trimethylamidoborane is another of Dr. Lee's involvements.

Dr. McLachlan is involved in collaborative research with the Institute for Pharmacology and Toxicology, University of Wurzburg, Germany; Department of Obstetrics/Gynecology, Duke University Medical Center; and Department of Comparative Medicine, Bowman-Gray School of Medicine. These collaborative projects involve a detailed exploration of transplacental toxicity of DES and other hormonally-active chemicals.

Dr. McLachlan and Dr. Korach take part in collaborative studies regarding X-ray crystallography of DES metabolites with Dr. William Duax, Medical Foundation of Buffalo.

Dr. Korach and Dr. Maydl are also involved in collaborative DES studies with the Institute for Pharmacology and Toxicology, University of Wurzburg, Germany.

Dr. Korach is involved in collaborative DES studies with Dr. Jack Kepler at the Research Triangle Institute.

Dr. Pratt is involved in collaborative studies with Dr. Ken Brown, National Institute for Dental Research; Dr. G. Cunha, University of Colorado Medical School; Dr. T. Yoneda, University of Osaka, Japan; and Dr. L. Dencker, University of Uppsala, Sweden.

Drs. Dixon, Lee, and McLachlan are also active in the US-USSR collaborative research programs in environmental health concerning transplacental toxicity and toxicology.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer recognition and scientific relevance of current Laboratory programs is the frequency that LRDT scientists organize and participate in "state of the art" symposia and are asked to serve on various committees attempting to provide meaningful directions in environmental helath 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-Elect of the Society of Toxicology, member of the Committee on Environmental Pharmacology of ASPET, Committee on Women in the Workplace organized by NIOSH, Chairman of the FDA Caffeine Study Review Panel, US-USSR Problem II Coordinator of the Joint Committee for Health Cooperation-Environmental Health involving chronic organ toxicity, Council of the Pan-American Medical Association Section on Environmental Health Sciences, Council for Agricultural Science and Technology, and the Toxicology Review Panel of WHO's Expanded Programme of Research Development and Research Training in Human Reproduction. He continues to be involved in the U.S.-Egypt Cooperative Agreement by participating in the Joint Working Group on Health Cooperation's Subcommittee on Envrionmental and Occupational Health. The Subcommittee is currently planning five workshops as the result of a Symposium on the Biomedical Impact of Technology Transfer held in Cairo, February, 1980. Dr. Dixon is also involved in the WHO/International Programme on Chemical Safety (IPCS) and has participated in two conferences supported by WHO/IPCS. In addition, Dr. Dixon has been co-organizer of several Target Organ Toxicity Symposia in cooperation with the Society of Toxicology. Proceedings of these Symposia are published in Environmental Health Perspectives and include symposia on liver, kidney, lung, gonads, development, nervous system, cardiovascular system, intestines, blood, endocrine system, and eye, ear, and other special senses. Dr. Dixon also organized a NTP/NIEHS Reproductive Toxicology Workshop. A working report from the Workshop represents the first step in the NTP/NIEHS effort to review reproductive toxicology.

Dr. McLachlan is a member of the DHHS Task Force on DES toxicity and has been advisor to NIOSH and the FDA on the toxicity of estrogens. He also served as a section coordinator for the NTP/NIEHS Reproductive Toxicology Workshop.

Dr. Pratt has been involved in the organization of an International Symposium on Current Research Trends in Craniofacial Development, International Symposium on In Vitro Screening Tests for Teratogenicity, and Environmental Protection Agency Meeting on Risk Assessment.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. LRDT scientists are frequently asked to review manuscripts for journals oriented toward Biochemistry, Pharmacology, Toxicology, and Teratology. Dr. Dixon is on the Editorial Boards of Environmental Health Perspectives, Toxicology and Applied Pharmacology, Journal of Pharmacology and Experimental Therapeutics, The Encyclopedia of Pharmacology and Therapeutics, Journal of Environmental Sciences and Health, Journal of Environmental Pathology and Toxicology, and Journal of Toxicology and Environmental Health.

Dr. McLachlan is on the Editorial Board of the International Journal for Biological Research in Pregnancy.

Dr. Pratt is on the Editorial Boards of Differentiation, Teratogenesis, Mutagenesis and Carcinogenesis, and Journal of Craniofacial Genetics and Developmental Biology.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Reproductive and Developmental Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training activities, including accepting adjunct appointments at nearby universities, supervising graduate student research, developing graduate courses in environmental health, and participating in the Fogarty International Center's Visiting Program.

Dr. Hall is an Adjunct Assistant Professor at the University of North Carolina School of Medicine, Department of Obstetrics and Gynecology.

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 Lee.

Drs. Hall, McLachlan, Korach and Pratt have lectured at UNC and/or Duke in areas of their research expertise.

Dr. Hall also lectured at the National Center for Toxicological Research and Sandoz Laboratories, Basel, Switzerland.

Dr. Pratt has organized a course at the University of North Carolina entitled "Developmental Toxicology and Teratology." Dr. Dixon is an invited lecturer for the course.

Dr. Pratt is an Adjunct Professor at the University of North Carolina in the Department of Anatomy.

Dr. Pratt has also lectured on Teratology for the Toxicology Program of the Triangle Area and at the University of Helsinki and the University of Uppsala, Sweden.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70010-05 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Developmental Disorders Using Cultured Embryos

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

PI:	R. M. Pratt	Research Chemist	LRDT	NIEHS
OTHER:	F. R. P. Sim	Visiting Associate	LRDT	NIEHS

COOPERATING UNITS (if any)

NIDR, NIH
Oak Ridge National Laboratories

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objectives of this project are: (1) To culture postimplantation embryos in order to aid the laboratory assessment of potential teratogens; (2) to define the mechanism of teratogenesis at the morphological, biochemical and molecular level using cultured embryos exposed to teratogens of interest.

(*Formerly, Study of Reproductive and Developmental Disorders Using Cultured Embryos)

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat embryos are cultured during organogenesis when they are most susceptible to teratogens. Conceptuses, dissected out of the mother on pregnancy days 9 or 10, are cultured in heat-inactivated homologous serum for 2-3 days at 38°C. Rotating bottles are used with a gas phase containing 10-40% O_2 and N_2. Growth promoting agents or teratogens are added to the culture medium to test their effect on embryonic differentiation. In vitro development of the embryo is evaluated using morphological (gross, histology and EM), biochemical (DNA, RNA, protein) and cytogenetic (karyotyping, sister chromatid exchange) parameters.

MAJOR FINDINGS AND PROPOSED COURSE: (1) In vitro development of rat conceptuses was unaffected by the addition of cyclophosphamide alone to the medium. However, addition of a combination of cyclophosphamide, NADPH and rat liver microsomes produced deleterious effects on embryonic development indicating metabolic conversion of cyclophosphamide into active components which presumably induce abnormal development.

(2) Exposure to ethanol retards growth and differentiation in cultured rat embryos during organogenesis. The development of untreated embryos is indistinguishable from growth in utero. These data suggest that the hypoplastic features of children born to chronically alcoholic mothers are due, at least in part, to a direct action of ethanol, which causes reduced embryonic cellular proliferation early in gestation.

(3) Exposure to jervine, a steroidal alkaloid which produces cranial-facial anomalies in vivo in the rodent, elicits a dose- and stage-dependent malformation in cultured embryos. This provides evidence that jervine acts directly on the developing embryo in vivo to produce malformations. The mechanism is under study.

The proposed course of this project involves developing procedures for in vitro growth and differentiation of embryos under more defined conditions. Effects of direct or indirect acting teratogens on the in vitro embryo development will be studied, and conditions for metabolic activation of environmental agents by microsomal MFO enzymes as well as the maternal involvement in the production of actual teratogens will be explored in-depth. Chemical induction of embryonic chromosomal aberrations and sister chromatid exchange on cellular differentiation and other cellular processes during organogenesis will be evaluated. This developmental toxicity model will be further developed and the effects of selected chemicals will be observed in vitro and correlated with in vivo effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Exposure to adverse environmental factors is presumably a major cause of developmental abnormalities in man. The laboratory prediction of teratogenic chemicals is unsure and mechanism of teratogenesis unclear. An in vitro system for culturing embryos would provide an opportunity to study cellular and molecular processes associated with normal and abnormal development. Such an in vitro model would

also be useful in predicting the toxic potential of environmental agents and in understanding the biological mechanisms by which chemicals may disrupt early development and produce birth defects.

PUBLICATIONS

Sanyal, M. K.: Development of the rat conceptus in vitro and associated changes in components of culture medium. J. Embryol. Exp. Morph. 58: 1-12, 1980.

Allen, J. W., El-Nahass, E., Sanyal, M. K., Dunn, R. L., Gladen, B., and Dixon, R. L.: Sister-chromatid exchange analyses in rodent maternal, embryonic and extra-embryonic tissues. Mutat. Res. 80: 297-311, 1981.

Sim, F, R. P., Matsumoto, N., Denny, D., Goulding, E., and Pratt, R. M.: Specific developmental defects induced by jervine in whole embryo culture. Teratology (In press).

Sim, F. R. P., Brown, K., Omnel, L., Keller, R., and Pratt, R. M.: Jervine induced malformations in the A/J and C57BL/6J mouse. Terat. Mut. Carcin. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70011-01 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Normal and Abnormal Craniofacial Development

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

PI:	R. M. Pratt	Research Chemist	LRDT	NIEHS
OTHER:	F. R. P. Sim	Visiting Associate	LRDT	NIEHS
	W. D. Willis	Biologist	LRDT	NIEHS
	E. H. Goulding	Biological Laboratory Technician	LRDT	NIEHS

COOPERATING UNITS (if any)
Dental School, University of Osaka, Osaka, Japan
Department of Toxicology, Uppsala University, Uppsala, Sweden
Dental School, University of British Columbia, Vancouver, Canada

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Growth and differentiation of craniofacial tissue is complex and involves multiple cell types interacting with each other and a number of hormones and growth factors. Of particular interest are steroids such as glucocorticoids, steroidal alkaloids such as jervine and epidermal growth factor (EGF). These agents are teratogenic at high doses whereas physiological levels of EGF and glucocorticoids are required for normal growth and differentiation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cortisone-induced cleft palate was obtained by administering s.c. cortisone (100 mg/Kg) acetate daily (days 11-14) to inbred A/J mice. At various times on days 13, 14, and 15 when the secondary palatal shelves are elevating and fusing, fetuses were removed and quick frozen in hexane at -70°C. Fetal heads were then cryostat sectioned in the coronal plane and sections evaluated and quantitated as to the extent of palatal shelf elevation and epithelial contact.

Jervine was administered (PO) at 100 mg/Kg on day 8 (plug day 0) to pregnant C57BL/6J mice. Embryos or fetuses were recovered at various intervals and evaluated for abnormal development. C^{14}-3-O-acetyl jervine was administered by the same route and at the same time. At various intervals up to 72 hours, the amount of label present in various maternal and embryonic tissues was determined. TLC separation of extracted tissues and body fluids was used to determine the extent of metabolism of jervine.

Human embryonic fibroblasts from the secondary palate were obtained from the American Type Culture Collection in Rockville, MD. Cells were grown in medium containing either 10% fetal calf serum or the latter was substituted for with various hormones and growth factor. At various intervals, the cell number, the incorporation of labeled thymidine and the activity of ornithine decarboxylase (ODC) were measured.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Cortisone treatment was found to delay the elevation of the palatal shelves for approximately 12-18 hours. After elevation occurred, the extent of epithelial contact between the shelves was minimal in the treated group and insufficient to maintain the epithelial surfaces in contact for subsequent fusion. These data provide convincing evidence that the mechanism of cortisone-induced cleft palate does not involve altered epithelial development, but is due to a direct effect on the ability of palatal shelf to obtain normal size and elevate on time.

(2) Jervine is a plant steroidal alkaloid which is shown in our studies to induce a number of malformations of the craniofacial region depending upon the strain of mouse, the dose and the time administered. When administered on day 8, clefts of the primary palate were predominant. Administration of labeled jervine indicated that either jervine or a metabolite cross the placenta and directly alter some aspect of normal growth and differentiation to produce craniofacial anomalies. Future studies are aimed at determining the morphological, biochemical and molecular basis for these observed effects.

(3) Human embryonic palatal mesenchymal cells (HEPM) were found to be a good model system in which to determine the factors regulating certain aspects of craniofacial development. These cells respond to pharmacological levels of glucocorticoids such as dexamethasone (DEX) by a pronounced inhibition of growth. This growth inhibition presumably is the major effect of cortisone responsible for cleft palate. These HEPM cells can be grown in a completely defined medium and our studies show that growth is highly dependent upon the presence of EGF, which is

thought to be involved in a fetal form in normal palatal development. These HEPM cells, which are established in culture appear to be an excellent model in which to further explore the biochemical and molecular basis for normal and abnormal craniofacial development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Facial clefting is a significant human congenital malformation and is one of the most frequently observed abnormalities. The etiology of facial clefting is complex, but appears to involve both genetic and environmental factors. The mouse is an excellent model since various inbred strains are exclusively sensitive to craniofacial teratogens. Our studies involve both whole animal as well as cell and organ culture to further define the complex interactions between genetic and environmental factors. Information from our studies may lead to better genetic counseling and drug avoidance during pregnancy and may reduce the frequency of congenital facial clefting in the human.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-05 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Mechanism of Androgen Mediated Gene Expression in Male Sex Accessory Glands

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

PI:	S. E. Harris	Senior Staff Fellow	LRDT	NIEHS
OTHER:	P. E. Mansson	Visiting Associate	LRDT	NIEHS
	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The objective of this project is to study the mechanisms involved in androgen mediated or estrogen influenced 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. The androgen induction of IV and V mRNA in castrate rats was measured using the respective probes. Mouse poly(A^+)-RNA IV was shown to cross-react with rat cDNA IV. The chromosomal genes for IV and V were isolated from a rat 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 very near a rat repetitive sequence. This insertional element may define an allelic difference in the SVS IV gene. The SVS IV and SVS V gene are linked and a detailed study of their 5'-flanking sequence is under way.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: E. coli. RR1 containing plasmids with SVS IV or V inserts were grown and amplified in L-broth containing tetracycline. Plasmids were isolated using CsCl-propydium iodide centrifugation. Restriction digest and agarose or acrylamide electrophoresis were 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 concentration was baked onto the filters. Probes of cloned DNA fragments were prepared with ^{32}P-NTP and nick-translation. Southern and Northern hybridizations were performed by standard techniques. Subcloning in pBR325 was performed by T4 ligation of EcoR1 fragments, chlormphenicol selection, and Grunstein-Hogness hybridization procedures. Phage clones were screened after amplificiation 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.

MAJOR FINDINGS AND PROPOSED COURSE: Molecular Characterization of Rat Seminal Vesicle Genes Whose Expression is Androgen Dependent. 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 has been determined. Using cDNA IV as probe, the induction of the messenger RNA for SVS IV was determined after testosterone administration to four week castrate rats. Interestingly, after four hours of hormone treatment, the level of mRNA IV increased approximately 30 fold. By 96 hours, the number of molecules of mRNA IV per seminal vesicle epithelial cell had gone from 24 to 100,000, a 4,000 fold increase. The cDNA IV probe was also used to hybridize to mouse poly(A^+)-RNA and a positive hybridization was obtained; we can, therefore, use the rat SVS IV probe to do a variety of experiments with mouse seminal vesicles treated prenatally and postnatally with various chemicals. By a dot blot assay, using cDNA to SVS V, the induction of mRNA V was determined and shown to be similar to the pattern obtained for mRNA IV. The cDNA IV and cDNA V do not cross hybridize with each other as we previously thought before pure cDNA V was obtained by molecular cloning. This finding led to an interesting observation (see below).

The cDNA IV probe was used to screen a rat library. Four of the original 11 signals have been purified and the phage DNA isolated. Restriction maps derived from stained gels and Southern blots were first developed, and then the EcoR1 fragments from three of the clones were subcloned into the EcoR1 site of pBR325. The fragments containing the entire SVS IV gene were further characterized by restriction mapping and DNA sequencing. The following conclusions have been reached. The entire SVS IV gene is on either a 3.3 kb or a 3.5 kb EcoR1 fragment. This 200 bp difference or insertion has been mapped to a position within the second intron of the SVS IV gene. The entire gene has a length of about 1.9 kb (in the 3.5) with the insertion somewhere between 1.25 and 1.65 kb from the 5'-initiation site. The 200 bp insertion seems to represent an allelic difference in the SVS IV gene since, (1) random bred Sprague-Dawley rats have both 3.3 and

3.5 kb EcoRl fragments and Fisher inbred rats have only the 3.5 kb fragment; and (2) the rat SVS IV flanking DNA sequences in the two λ clones which contain either the 3.3 kb or the 3.5 kb EcoRl fragment are identical. This insertional element also seems to be unstable in the phage since both the 3.3 kb and 3.5 kb EcoRl forms were derived from one original signal in the entire gene library. The phage was plaque purified, and again the flanking sequences around the 3.3 kb or 3.5 kb EcoRl fragments are identical. Also a repetitive DNA sequence(s) is also closely associated with the insertion and may be intimately involved in the insertional instability. Finally, one of the λ clones was shown to hybridize to both a cDNA IV probe and a cDNA V probe, indicating that the SVS IV and SVS V are closely linked within the rat genome. This clone has been purified and the DNA is presently being characterized. Our initial observation is that the natural SVS V gene is on a 6 kb EcoRl fragment. The EcoRl fragments for SVS V have been subcloned. This linkage of two genes whose expression is androgen dependent opens up an interesting and exciting possibility. A detailed study of the 5'-flanking sequences may reveal some conserved DNA regions which play some role in androgen action.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Using the technology presently available, a relatively detailed model of how steroid hormones work 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

Mansson, P. E., Silverberg, A. B., Gipson, S. H. and Harris, S. E.: Purification of the major abundance class of poly(A^+)-RNA from rat ventral prostate. Mol. Cell. Endocrinol. 19: 229-241, 1980.

Pan, Y. C., Silverberg, A. B., Harris, S. E. and Li, S. L. : Complete amino acid sequence of a major secretory protein from rat seminal vesicle. Int. J. Peptide Protein Res. 16: 143-146, 1980.

Carter, D. B., Silverberg, A. B. and Harris, S. E.: The effect of spironolactone on androgen dependent proteins of rat ventral prostate. J. Endocrinol. 86: 471-476, 1980.

Newbold, R. R., Carter, D. B., Harris, S. E. and McLachlan, J. A.: Molecular differentiation of the mouse genital tract: Serum free organ culture system for morphological and biochemical correlations. In Vitro 17: 51-54, 1981.

Carter, D. B., Silverberg, A. B., and Harris, S. E.: Effect of testosterone propionate on protein synthesis by two-dimensional electrophoresis in rat ventral prostate. Arch. Androl. 6: 133-140, 1981.

Mansson, P.-E., Sugino, A., and Harris, S. E.: Use of a cloned double-stranded cDNA coding for a major androgen dependent protein in rat seminal vesicle secretion: The effect of testosterone in gene expression. Nucleic Acid Res. 9: 935-946, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70047-05 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Prostate as a Model System to Study Normal and Abnormal Gene Expression in the Rat and Human

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

PI:	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS
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	S. E. Harris	Senior Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

None

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Three clones for structural genes coding for proteins secreted from the rat ventral prostate have been identified by hybrid arrest translation. The three cloned inserts have been mapped with restriction endonucleases, and the homologous mRNAs have been shown to be induced by androgens. A rat gene library carried in λ phage charon 4A is currently being screened for the genomic sequences corresponding to two of the secreted proteins. In addition, total poly(A^+)-mRNA from human prostate tissue has been cloned using the E. Coli-PBR322 system. These clones are currently being screened for the structural gene corresponding to a major secretory protein previously identified by two-dimensional gel electrophoresis of human prostatic fluids.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Poly(A^+)-mRNA from rat ventral prostate was fractionated by SDS-sucrose gradients and double-stranded complementary (cDNA) DNA was synthesized using reverse transcriptase for both strands. After "tailing" the cDNA with deoxyadenosine residues, the mixture was annealed to dT tailed plasmid PBR322.

Total poly(A^+)-mRNA was isolated from human benign hyperplastic tissues and double-stranded cDNA was synthesized as above. The cDNA was tailed with deoxycytidine residues and then annealed with dG tailed PBR322.

E. Coli RR1 were transformed with the recombinant plasmids, and ampicillin sensitive/tetracycline resistant clones were selected for plasmid purification. Hybrid arrest translation of total poly(A^+)-mRNA hybridized with restriction endonuclease digested plasmid DNA isolated from various rat clones was used to identify three plasmids containing the coding information for three rat prostate secretory proteins. The E. Coli clones containing human structural genes were characterized by isolation of plasmids and sizing of inserts on agarose gels.

MAJOR FINDINGS AND PROPOSED COURSE: Restriction maps of cloned structural genes for three of the major secretory proteins from rat ventral prostate have been obtained. Purified inserts from these plasmids have been obtained from agarose gels. Nick-translated probes have been synthesized from the pure inserts and used to follow the androgen regulation of the homologous mRNA's in the rat ventral, lateral, and dorsal prostates. In addition, nick-translated probes to two of the inserts have been used in a primary screening of the rat gene library carried in λ phage charon 4A. The natural genes for these two androgen dependent secretory products will be isolated by repeated rounds of screening until the genomic sequences are obtained.

The human structural gene library derived from the benign hyperplastic tissue has been characterized thus far by sizing of inserts. The structural gene coding for the major low molecular weight secretory protein (15,000-17,000 daltons) will be preliminarily identified by hybrid-selection translation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The rat ventral prostate is dependent on androgen for normal secretion and function. This organ has a set of genes which produce large quantities of particular messenger RNAs and their corresponding structural proteins, thus making this system ideal for studying the molecular mechanisms of androgen mediated gene expression.

This basic knowledge and technology will then be applied to the human prostate and its abundant messenger RNAs. Potential markers for normal, as well as abnormal prostatic tissue, can then be developed. Prostate disease in humans is of high incidence and does have an environmental component. The markers we develop may, therefore, be of some usefulness in a clinical setting.

PUBLICATIONS

Carter, D. B., Silverberg, A. B. and Harris, S. E.: The effect of testosterone propionate on protein synthesis in rat ventral prostate. Arch. Androl. 6: 133-140, 1981.

Carter, D. B., Silverberg, A. B. and Harris, S. E.: Effect of spironolactone on androgen-dependent proteins in the ventral prostate of the rat. J. Endocrin. 86: 471-476, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70060-08 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Development and Function

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	2.8	1.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The long-range goals of this project are: (1) to evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring; (2) to investigate the mechanisms involved in the production of subfertility in mammals as a result of their in utero exposure to foreign chemicals; (3) to assess the transplacental carcinogenic potential of these compounds; (4) to study the physiologic disposition and metabolism of these compounds in the pregnant animal and fetus; (5) to study chemico-biological interactions of transplacental toxicants, with special emphasis on structure-activity relationships; (6) to determine if prenatal exposure to environmental agents can alter the biological response to steroid hormones in reproductive tract tissues; (7) to develop and utilize organ culture systems to study the effects of environmental chemicals on the development of the fetal reproductive tract in vitro; and (8) to evaluate the above animal models as predictors of human response. Special attention is given to diethylstilbestrol (DES).

PROJECT DESCRIPTION

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the function status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, and other parameters of function. Pharmacological, biochemical, physiological, and morphological procedures were used which included polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, inverse isotope dilution analysis and chromatography, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS AND PROPOSED COURSE: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock growth promotor and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring. Continuation of these studies has shown that such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma, squamous cell tumors of the vagina and ovarian tumors. A common non-neoplastic lesion of the DES-treated mouse was squamous metaplasia of the uterus. This abnormality was determined to require a secondary stimulus by estrogen at puberty for its manifestation. Anatomical changes such as cervical enlargement without luminal size changes, altered utero-tubal junctions, and uterine shape changes may be important to understanding reported subfertility in similarly exposed women. Stromal hyperplasia of the vagina, cervix and uterus has raised the question of the role of this tissue component in the observed lesions; the demonstration of uterine and cervical stromal sarcomas in prenatally DES-treated mice further emphasizes the importance of studies on stromal-epithelial interactions during abnormal development of the genital tract. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β-estradiol. Differential fetal protein binding of DES and estradiol may help explain these results. Bioavailability at many levels may be a determining factor in the transplacental toxicity of hormonally-active xenobiotics. Moreover, the stage of cell differentiation at the time of DES exposure is critical in determining the type of lesion expressed later in life. DES treatment during early cytodifferentiation of the female genital tract is associated with vaginal adenocarcinoma but very little vaginal adenosis; treatment during later cytodifferentiation of this tissue is associated with adenosis but not vaginal adenocarcinoma. Ovarian abnormalities in morphology and steroid secretion in prenatally DES treated mice appear to arise from altered differentiation of the fetal ducts which contribute to ovarian morphogenesis.

In continuation of studies with male offspring from DES treated mice, the fate of Müllerian duct remnants in DES treated males was determined and the role of these tissues in prostatic lesions evaluated. These remnants were shown to respond to estrogen with typical Müllerian features. Müllerian remnants were observed in treated male offspring at any stage of development. Alterations in these tissues

were, in part, related to changes in estradiol and Müllerian inhibiting factor (MIF). Most abnormalities observed in the genital tracts of exposed men and women have been duplicated in our mouse model. Additional alterations seen in the mouse may be predictive for the human. In both male and female offspring, attempts will be made to establish biochemical/morphological markers for genital tract lesions. For example, the SDS protein profile of the secretions of the prostate and seminal vesicles of DES mice were shown to be altered; castrate males exposed prenatally to DES and postnatally to estradiol had seminal vesicle secretory patterns identical to uterine luminal fluid.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g, β-dienestrol and ω-hydroxy DES) were identified in the mouse fetus and neonate exposed to ^{14}C-DES. Recently, organ cultures of the fetal mouse genital tract have been shown to oxidatively metabolize DES. Studies on the bioactivation of DES have shown the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from target tissue, the mouse uterus. DES was shown to be oxidatively metabolized during prostaglandin synthesis by the peroxidase activity of prostaglandin synthetase from sheep seminal vesicle microsomes. The role of this activity in target organ bioactivation remains to be established. The estrogenic activities of a series of DES metabolites and analogs were determined. Results suggest that DES metabolism follows alternative pathways resulting in metabolites which retain estrogenicity or those in which such activity is absent. These studies have been expanded with special emphasis on the biological significance of potentially activated metabolites; such data should aid in generalizations to other classes of estrogenic environmental chemicals. In addition, studies of DES metabolism in target/non-target tissues and in cell culture are being continued. For example, it was shown that peroxidase, an enzyme inducible in estrogen target tissue, is able to metabolize DES to its major metabolite, β-dienestrol. Fluorinated derivatives of DES have been made to help assess the role of metabolism in toxicity. These studies are augmented by experiments on the in vitro metabolism of DES by a transformable cell system in which conversion of DES to β-dienestrol was demonstrated. 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.

Studies in organ culture have shown that DES can alter normal differentiation of the genital tract in vitro. These studies will be continued to evaluate the role of organ/organ and cell/cell interactions in genital tract development. Thus, DES has been shown to alter the action of MIF on the in vitro differentiation of the Müllerian ducts. Two-dimensional gel electrophoretic maps of the protein changes during organogenesis of the female genital tract have been developed to aid in an understanding of the molecular events which determine normal or abnormal differentiation of this system. Also, experiments utilizing the separation and recombination of stroma and epithelium of DES treated fetal reproductive tracts have been undertaken to determine the role of such tissue interactions in DES induced genital abnormalities.

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.

PUBLICATIONS

Lamb, J. C. IV, Newbold, R. R. and McLachlan, J. A.: Evaluation of the transplacental toxicity of diethylstilbestrol with the scanning electron microscope. J. Toxicol. Environ. Health 5: 599-603, 1979.

McLachlan, J. A., Baucom, K., Korach, K. S., Levy, L. and Metzler, M.: A novel fluorinated derivative of diethylstilbestrol. Steroids 33: 543-547, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-05 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity

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

PI:	K. S. Korach	Research Endocrinologist	LRDT	NIEHS
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	L. Levy	Research Chemist	ECB	NIEHS
	V. B. Quarmby	Visiting Fellow	LRDT	NIEHS

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Environmental Chemistry Branch, NIEHS

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Transplacental Toxicology Group

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The main objectives of this project are to determine whether DES is metabolized to biologically and hormonally active metabolites; to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins particularly alpha-fetoprotein; to investigate some of the biochemical mechanisms which contribute to effects of prenatal exposure of mice to hormonally active environmental chemicals, to investigate the mechanism of uterine hormonal responsiveness and to determine the molecular locus of transplacental toxicity using structure-function relationships of different environmental chemicals; and to determine biochemical markers for transplacental toxicity. These objectives are approached using refined biochemical techniques of hormone receptors and hormone action. The basic physiological effects on hormone synthesis and hormone levels will be studied using chemical extraction techniques and radioimmunoassays. The carcinogenic nature of hormonally active environmental chemicals will be studied in vivo.

PROJECT DESCRIPTION

METHODS EMPLOYED: Hormonal effects were studied with receptor binding techniques including saturation binding, competition studies, Scatchard plot analysis, sucrose gradient centrifugation, glucose oxidation/utilization, RNA polymerase enzyme activities, extraction of nuclear DNA polymerase enzymes and DNA synthesis quantitation, 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 were examined. Characterization of uterine nuclear estrogen receptors in the mouse has indicated two forms of the receptor (analogous to Type I, II reported in rat). Presently, the best experimental conditions for studying these receptor forms are being determined. Results have shown that those animals in the non-responsive 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 5 through 40 day old animals have been measured. These studies suggest that at 5 days of age this pattern of receptor differences is already apparent. Studies to determine receptor levels in fetal reproductive organs are being planned as well as the development of a microsteroid receptor assay. 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 difference in cytosol receptor levels could not be explained by differential accumulation of receptors in the nucleus since assays of nuclear receptor in these same tissues showed no differences.

Receptor differences in control and DES treated offspring were also found in studies demonstrating the responsiveness of the receptors to estrogen administration. The mouse uterus possesses a second translocation of hormone receptor complex to the nucleus after exposure to hormone. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear peak. Steroid autoradiography techniques were used to demonstrate that the two events are occurring in different uterine cell types. There is a temporal pattern of interaction with the hormone appearing in the nuclei of stromal and glandular epithelial cells and later in luminal epithelium. The mechanism for this differential interaction is being investigated in more detail. The role of this event in estrogen action in the mouse reproductive tract, with particular regard 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 polymerase 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 a 44,000 mw protein in uterine tissue from hyperestrogenized animals. A 69,000 mw protein was also found in the incubation media from these same tissues. Incubations with uteri from control animals did not show the presence of these proteins. Attempts will be made to produce a poly(A^+)-mRNA preparation and library to study the mechanism of their synthesis.

In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo bioassay and an in vitro receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity; while certain metabolites, e.g. β-dienestrol or ω-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation. The exception to these results were some indenestrol isomers and ψ-DES, which are possible DES metabolites and which show receptor binding comparable to DES, but were 20-100 times less biologically active. These studies have suggested a mechanism of altered/differential clearance of these analog receptor complexes from the target cell nucleus.

Studies of receptor and plasma binding activities, particularly to alpha fetoprotein, of various DES analogs and metabolites will be continued to determine the structural site of chemicals exhibiting hormonal and/or carcinogenic actions. These structural requirements were exemplified by studies determining estrogen mitogenic activity of the DES compounds. Two of the indenestrol isomers, differing only in the position of a double bond, showed divergent uterine DNA stimulation. A unique DES derivative containing fluorine atoms has been synthesized and its hormonal activity is being tested. Preliminary studies indicate the compound interacts with receptor binding sites in a biphasic mechanism and that this complex can translocate to uterine cell nuclei. Tests with this compound using additional biochemical assays are underway.

Binding studies are being expanded to allow the potential hormonal activity of selected environmental chemicals to be determined in 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 compounds 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 subjects 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 in utero environmental chemical effects on the reproductive system of the offspring is so limited, these studies will help recognize 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.

PUBLICATIONS

Korach, K. S., Fox-Davies, C. and Baker, V.: Differential response to estriol and estradiol in the mouse uterus: Correlation to an additional nuclear event. Endocrinology 106(6): 1900-1906, 1980.

Korach, K. S.: Biochemical and estrogenic activity of some diethylstilbestrol metabolites and analogs in the mouse uterus. In Leavitt, W. W. (Ed.): Hormones and Cancer, Plenum Press (In press).

McLachlan, J. A., Newbold, R. R., Korach, K. S., Lamb, J. C. and Suzuki, Y.: Transplacental toxicology: Prenatal factors influencing postnatal fertility. In Kimmel, C. and Buelke-Sam, J. (Eds.): Developmental Toxicity, New York, Raven Press (In press).

Korach, K. S.: Selected biochemical actions of ovarian hormones. Target Organ Toxicity: Endocrine Systems, Environ. Health Perspect. (In press).

Korach, K. S., Harris, S. E. and Carter, D. B.: Uterine proteins influenced by estrogen exposure: Analysis by two-dimensional gel electrophoresis. Mol. Cell Endo. (In press).

Korach, K. S. and Lamb, J. C.: Estrogen action in the mouse uterus: Differential nuclear localization of estradiol in uterine cell types. Endocrinology (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70080-08 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

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

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	R. Bechter	Visiting Fellow	LRDT	NIEHS
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Department of Reproduction and Population Dynamics, Johns Hopkins University

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Toxicology Group

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 (200 words or less - underline keywords)

These studies seek to assess the effects of environmental agents on spermatogenesis, male accessory glands, and male 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 on-going: (1) species differences in DNA repair capability have been identified and the underlying biological processes which account for such differences are being sought; (2) the biotransformation of polycyclic aromatic hydrocarbons by cell free testicular homogenates and microsomes are being compared qualitatively and quantitatively to results obtained with the isolated perfused testis preparation; (3) the mechanisms by which DNA-damaging alkylating agents increase the TCDD induction of aryl hydrocarbon hydroxylase activity in the prostate gland 5 fold are being investigated; (4) the effects of gestational exposure to TCDD on the testicular development and reproductive performance of male offspring are being described; and (5) quantitative radioautographic studies using computer assisted image enhancing is being used to further define the blood-testis barrier.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) DNA Repair. Differential testicular DNA repair activity was investigated in spermatogenic cells of various mouse strains. Prespermiogenic cells were isolated following collagenase trypsin digestion and enriched by albumin-gradient centrifugation. Enriched cells demonstrated a viability greater than 95% by trypan blue exclusion criteria. Unscheduled DNA synthesis (UDS) was determined in vitro by incubating 10^6 cells/ml with methylmethane sulfonate (MMS), 0.4 mM, in the presence of hydroxyurea (20 mM).

(B) Perfusion of isolated rat testis. Adult Sprague-Dawley rats were treated with a single oral dose of TCDD (10 µg/kg) 72 hours before the animals were killed and the testes quickly excised. The testicular artery was cannulated with an 80 micron glass capillary tube attached to polyethylene tubing and perfused at a rate of 13.3 ml/g/hr with an oxygenated Krebs-Ringers bicarbonate solution containing 3% bovine serum albumin. Benzo(a)pyrene (BP) C^{14}-labelled benzo(a)pyrene (BP) at a concentration of 3.6×10^{-6} M was added to the perfusate and BP metabolites determined in the organic extractable phase using a combination of differential solvent extractions and high-pressure liquid chromotography (HPLC).

Preparation of testicular homogenates. Sprague-Dawley rats (CD strain) were sacrificed by cervical dislocation and the testes immediately excised, weighed, and homogenized, using a Potter-Elvehjen vessel fitted with a teflon pestle, in 4 volumes of ice cold 0.15 M KCl in 0.02 M HEPES at pH 7.4. Each reaction vessel contained 0.25 mM HEPES (pH 7.4), 280 pmol radiolabelled BP, 870 nmol glucose-6-phosphate, 220 nmole NADP, 870 nmole $MgSO_4$ and 25 mg of testicular homogenate per ml. A total volume of 2 ml was incubated for 30 minutes at 32^{o} C. The reaction was stopped by addition of two volumes of ethyl acetate/acetone (2:1), extracting three times with vigorous shaking, and the organic extract processed for HPLC analysis.

(C) 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 triethylenemelamine (TEM)). The DNA damaging agents were administered intraperitoneally 24 hours prior to oral treatment with TCDD (10 µg/kg). Hepatic and prostatic microsomes were prepared by homogenizing tissue in 4 volumes of ice cold 0.15 KCl in 0.02 M HEPES, pH 7.4. The homogenate was centrifuged for 15 minutes at 9,000 x g at 4^{o}C. The supernatant was then removed and centrifuged at 176,000 x g for 45 minutes to obtain microsomes and soluble fraction. The microsomal pellet was resuspended in KCl-HEPES and centrifuged again. The washed microsomes were then suspended again in KCL-HEPES. AHH activity was assayed by measuring the fluorescence of alkali-extracted BP phenols with a Aminco Bowman spectrofluorometer using 3-OH BP as a standard.

(D) Effects of prenatal exposure to TCDD on the testicular development and reproductive capacity of male offspring. Pregnant Sprague-Dawley female rats were treated on days 10 to 13 of gestation with daily oral doses of either corn oil or 0.5, 1.0, or 2.0 µg/kg of TCDD. Pregnant rats were allowed to deliver

their young and raise them until weaning. When the male offspring were 70 days old, testicular function was estimated by measuring plasma FSH, LH, and testosterone. Testicular weights were recorded and seminiferous tubule diameters and histopathology were determined using light microscopy.

(E) In vivo assessment of fertility. Fertility was assessed using the serial mating technique. Males were housed with untreated virgin female CD rats at 7-day intervals for 10 weeks. Daily examination of vaginal plugs for the first two weeks was carried out to assess libido and determine mating capability. One week after the females were removed from the males, they were killed and the total number of implants (alive and dead) was scored.

(F) Studies of the blood-testis barrier using quantitative autoradiography with image enhancing. Male CD rats were anesthesized and C^{14}-labelled sucrose or α-aminoisobutyric acid (AIB) infused via femoral vein for 60 min. Blood levels of sucrose and AIB were monitored. At the end of the infusion, the testes were immediately frozen in nitrogen and then sectioned (20 μ) with a cryostat. The tissues were dry mounted and exposed to X-ray film for 30 days. C^{14} activity in the testicular section is being quantitated using an image analyzer system coupled with a PDP 11 computer and Sokolow C^{14}-standards.

MAJOR FINDINGS AND PROPOSED COURSE: (A) DNA Repair. Significant strain differences were found when DNA repair by testes of immature mice (20 days old) was studied. MMS-induced repair activity in the CD-1 mouse was linear for up to 4 hours and dose dependent. The apparent K_m for MMS-induced repair activity in prespermiogenic cells was 1.8×10^{-4} M. Of the mouse strains tested, C57Bl/6J demonstrated the highest activity while BALB/CJ exhibited the lowest. A maximal difference of about 3.5 fold was observed between these two strains. On-going studies seek to determine if the differences in DNA repair activity are due to differential DNA repair enzyme activities, thymidine pool sizes, differential thymidine kinase activity or permeability. The efficiency of alkylated purines and pyrimidines removal is being determined by analyzing DNA of sperm taken from the epididymides. Exonuclease activity and overall DNA repair enzyme activity are also being assessed.

(B) HPLC patterns of BP metabolites in the perfusate and tissue of perfused testis. The 9,10- and 7,8- positions of BP are preferentially epoxidated compared to liver. Since 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene is thought to be the precursor of a potent carcinogen, the TCDD-induced shift in metabolic products could be an important determinant in tumor induction. The ratio of water soluble to organic soluble metabolites in cell-free homogenates and the perfused testis were 1.1 and 3.0 respectively. Specific conjugating enzymes appear to be more active in the intact testis. Furthermore, the magnitude of various metabolites in the homogenate ranges from 3.5 to 164 times that determined in the perfused testis. The total BP metabolites in the homogenate of either the control or TCDD-induced testis were 16 times that of the intact organ. Thus, BP metabolism in the cell free system differs both qualitatively and quantitatively from results obtained with the perfused male gonad.

(C) 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 treatment intraperitoneally with DNA damaging agents. Ongoing studies seek to determine if the alkylating agents permit greater TCDD interactions with sites on the DNA (Ah locus) and, thus, increases the rate of transcription. Using cDNA probes, mRNA levels are being determined following the combined treatment with alkylating agents and TCDD and compared to treatment with TCDD alone.

(D) Effects of prenatal exposure to TCDD chemicals on the testicular development and reproductive capacity of male offspring. Prenatal exposure to TCDD resulted in dose-related abnormal testicular development in certain male offspring. A dose-related decrease in litter size associated with exposure to TCDD during gestation was noted. Birth weights were also decreased and testes were smaller. The testes were smaller and had reduced diameters of seminiferous tubules.

(E) In vivo assessment of fertility following prenatal TCDD treatment. Serial mating of male offspring exposed during gestation to TCDD demonstrated a decrease in the number of live offspring and an increase in the number of dead implants. However, because these effects were only associated with selected offspring, the differences were not statistically significant. These data are being analyzed to determine if gestational TCDD exposure alters the ability of certain male offspring to produce normal numbers of viable offspring due to genetic effects on the spermatogenic stem cells.

(F) Quantitative autoradiographic studies of the blood-testis barrier (BTB). Preliminary observations suggest that computer image enhancement of testicular autoradiographs is a useful tool to better describe the blood-testis barrier. However, resolution using C^{14}-labelled chemicals is insufficient to allow a detailed cellular characterization of the barrier. Efforts are underway to apply the technique using other radioisotopes, such as tritium, which offer increased resolution. Cells which constitute the barrier and factors which affect the BTB will be further studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Toxicological studies of a target organ, such as the testis, 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 the 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. DNA repair is a sensitive indicator of chemically damaged spermatogenic cells. In addition, the repair capability of male germ cells affords the organism some degree of protection against toxic agents. Chemicals such as benzo(a)pyrene are both activated

and detoxified by enzymes in testis and prostate glands. Differences in the mixed function oxidase(s) and the differential inducibility in these organs can significantly modify organ toxicity. The study of the role of biotransformation in the testis involves in vitro subcellular fractions, perfused organs, and whole animal approaches.

Improved short-term tests are essential to modern toxicology. Prediction of reproductive toxins is especially difficult. Understanding the pharmacokinetic characteristics of the BTB, toxication and detoxication mechanisms, as well as accurate assessment of germ cell DNA damage, will allow a better understanding of species differences with regard to reproductive and genetic toxicity. Such studies will increase the reliability of extrapolating laboratory animal data to man and subsequently estimating human risk.

PUBLICATIONS

Dixon, R. L. and Lee, I. P.: Metabolism of benzo(a)pyrene by isolated perfused testis and testicular homogenate. Life Sci. 27: 2439-2444, 1980.

Lee, I. P. and Suzuki, K.: Induction of aryl hydrocarbon hydroxylase activity in the rat prostate glands by 2,3,7,8-tetrachlorodibenzo-P-dioxin. J. Pharmacol. Exp. Ther. 215: 601-605, 1980.

Lee, I. P. and Nagayama, J.: Metabolism of benzo(a)pyrene by the isolated perfused rat testis. Cancer Res. 40: 3297-3303, 1980.

Shoji, R., Suzuki, K., and Lee, I. P.: Aggregation patterns of fetal rat brain cells following exposure to x-irradiation. Teratogenesis, Carcinogenesis, and Mutagenesis 1(3): 333-342, 1980.

Tyrer, H. W., Cantrell, W. T., Horres, R., and Lee, I. P.: Benzo(a)pyrene metabolism in mice exposed to diesel exhaust. I. Uptake and distribution. Proc. Intern. Symposium: Health Effects of Diesel Emissions, Vol. 1, pp. 508-519, 1980.

Lee, I. P. and Suzuki, K.: Differential DNA repair activity in prespermiogenic cells of various mouse strains. Mutation Res. 80: 201-211, 1981.

Suzuki, K. and Lee, I. P.: Induction of aryl hydrocarbon hydroxylase and epoxide hydroxase in rat liver, kidney, testis, prostate glands, and stomach by a potent nematocide, 1, 2-dibromo-3-chloropropane. Toxicol. Appl. Pharmacol. 58: 151-155, 1981.

Lee, I. P. and Suzuki, K.: Studies on the male reproductive toxicity of Freon 22. Fund. Appl. Pharmacol. (In press)

Lee, I. P., Suzuki, K., and Nagayama, J.: Metabolism of benzo(a)pyrene in rat prostate glands following 2,3,7,8-tetrachlorodibenzo-P-dioxin exposure. Carcinogenesis (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70085-04 LRDT

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of In Vitro Models for Assessing Reproductive Toxicity

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

PI:	J. L. Hall	Research Physiologist	LRDT	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LRDT	NIEHS

COOPERATING UNITS (if any)

None

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

Interspecies (human sperm/hamster ova) in vitro fertilization test results were compared with pregnancies and other clinical data of couples consulting a fertility clinic. Initial data confirm this test to be a valid and useful adjunct to semen analysis as tests of male fertility. The demonstrated cryopreservation of both sperm and ova greatly extends the scope of this test. A model computer program to analyze reproductive changes related to environmental factors is being established.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Usable animal models are urgently needed to identify the causative factors in human male fertility. Studies to test the validity of the interspecies fertilization system in the assessment of human infertility were continued. Improvements of the test-system were also of interest.

A. Of ultimate importance is the validity of the interspecies in vitro fertilization test-system as an accurate measure of human sperm function. Eighty infertile couples attending a fertility clinic agreed to participate in the study. A standard infertility evaluation was performed on each couple. A group of 30 normal men who had fathered children also participated in the study. Semen for each male was evaluated for volume, liquification, viscosity, color, pH, total sperm count, concentration (sperm density), progressive motility at 1 hr, motile sperm count, percent dead and percent abnormal forms. Sperm were prepared so that each fertilizing dish contained approximately equivalent numbers of motile sperm. Ova for fertilization were collected from Golden hamster oviducts by superovulation; they were denuded and incubated in vitro with sperm suspensions from either fertile or suspected infertile men. After three hours, ova were examined by phase-contrast microscopy for morphological evidence of fertilization. In order to determine the validity of this in vitro test, pregnancies for participants in the study are being followed.

B. Since toxicologic testing in the future will necessitate the use of semen samples collected at locations distant from "fertilization" test centers, semen samples from normal men were evaluated for their ability to withstand freezing and thawing. The semen was mixed with glycerol to a final ratio of one part glycerol to nine parts semen. The sample was cooled at a rate of 1-2°C per minute for 25°C to 0°C and 5-6°C from 0° to -30°C and then stored in liquid nitrogen at -196°C. After one week the semen sample was evaluated for the standard parameters and for sperm fertilizing capacity using freshly collected zona-free hamster ova.

C. Storage of ova would offer additional improvement to the test-system. Ova would be available to test sperm on short notice; and cryopreserved ova could be used when hormonally-treated animals do not produce sufficient numbers of fresh ova. Experiments were carried out to validate whether liquid nitrogen-preserved hamster ova could be substituted for fresh ova in the assessment of human sperm fertilizing capacity. Ova were frozen similarly to sperm and stored in liquid nitrogen vapor (-196°C) for one week to six months. Six different combinations of Fetal Calf Serum (FCS) and Dimethyl Sulfoxide (DMSO) were evaluated for optimal preservation of viability and fertilizability of ova.

MAJOR FINDINGS AND PROPOSED COURSE: A. For years it was accepted that male infertility was the result of a deficiency in sperm motility, sperm number, normal sperm morphology or a combination of semen inadequacies. Recent findings in this study, however, demonstrated that a deficiency in an intrinsic factor, sperm fertilizing capacity, may exist even when all or most of the standard parameters are normal. Since the conventional semen analysis currently used for

predicting male fertility is not totally reliable, we investigated the correlation of interspecies in vitro fertilization results (based on the penetration of zona pellucida-free hamster ova by human sperm) with clinical evaluation of the infertile couple. In vitro fertilization results in the 154 males evaluated correlated well with clinical diagnosis. In couples with a male factor, based on clinical history or semen analysis, the mean fertilization rate was 9%. The mean rate of fertilization in infertile couples with no known male factor was 40%. The mean penetration rate for all patients tested was 44% (0-100%). This was well below a mean fertilization rate of 67% (20-100%) for a group of normal donors. In addition, one-fifth of the patients fertilized no eggs at all, a phenomenon not observed in the donor population, and 50% of those patients who fertilized no hamster ova in vitro were clinically normal males. No subsequent pregnancies have occurred in this group. In contrast, all 21 of 23 couples presently with pregnancies or delivering during the study gave positive fertilization results in the in vitro test-system. Although we cannot morphologically distinguish between "fertile" and "infertile" sperm, the data pertaining to the validity of this test suggest that we can more accurately assess the fertilization potential of a particular sample without having to wait on the outcome of pregnancy.

B. In the 200 years since sperm were observed to survive freeze preservation, many improvements have been made in cryopreservation methodology which allow successful clinical use of frozen specimens. However, accurate identification of human semen samples which will withstand freezing and thawing is a serious problem with sperm storage today. From results with "normal" men there were only minor decreases in the standard clinical parameters caused by freezing and thawing. However, freezing and thawing had adverse consequences on the fertilizing capacity of several samples. The motility recovery rate after one week of storage at -196°C averaged 84% (72-100%). Although the motility of frozen-thawed sperm at 18-24 hours post-collection was equivalent to that of fresh, the recovery of fertilizing capacity was quite varied (0-100%) and not easily predicted. Prefreeze and post-thaw sperm yield mean fertilization rates of 65% and 47%, respectively. None of the standard semen parameters could identify specifically which individual sperm sample could not recover its fertilizing capacity. Likewise no assurance could be given that a particular sample would fertilize well after freezing. The reduced in vitro fertilizing capacity of actively moving sperm demonstrate that the latent cryoinjury may be due to impairment of the ability for sperm-egg fusion and not motility changes.

C. In contrast to sperm, animal ova were remarkably resistant to freeze-thaw damage. The percentages of ova intact or not damaged by cryopreservation were 96, 92 and 88% when stored in 20% FCS plus 0.5, 1.0 and 1.5 M DMSO, respectively. In the 10% FCS group the percentages of intact ova were 96, 94 and 82% for 0.5, 1.0 and 1.5 M DMSO, respectively. In comparision of fertilizability of stored ova, human sperm were found to penetrate 92, 91 and 86% of the ova in the 0.5, 1.0 and 1.5 M DMSO-20% FCS groups, respectively. Fertilization rates in the 10% FCS groups were 90, 89 and 88% for 0.5, 1.0 and 1.5 M DMSO, respectively. The mean fertilization rate for freshly collected ova not stored in FCS or DMSO was 94%. The data demonstrate that zona-free hamster ova can be successfully stored at ultralow temperatures (-196°C) for up to six months. Ova stored in low DMSO

and high FCS consistently gave better results and those ova were not greatly different from fresh ova in fertilizability or morphological appearance.

In the future we hope to improve sperm storage to the point that abnormal semen can be transported from distant occupational sites for testing and without loss of fertilizing ability. The correlation of in vitro fertilization results and pregnancies will be continually monitored in order to further assess the validity of using substitute ova. Furthermore, functionally deficient sperm samples will be examined for ultrastructural changes and for lectin binding to identify possible membrane changes related to loss of fertilizing capacity. Importantly, all male data (including occupational exposure and in vitro fertilization results) will be analyzed with computer assistance for an accurate perspective of environmental influences on human reproductive parameters.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human-hamster interspecies in vitro fertilization model developed here at the NIEHS should provide a step forward for evaluation of the relationship between human reproductive health and the environment. Improved aspects of this test-system described above will certainly broaden the capabilities of this test for use in future studies.

PUBLICATIONS

Hall, J. L.: Relationship between semen quality and human sperm penetration of zona-free hamster ova. Fertil. Steril. (In press).

Hall, J. L.: Effects of in vitro fertilization and manipulation of embryos on congenital malformations. Obstetrical and Gynecological Survey (In press).

Dixon, R. L., Sanyal, M. K., Kitchen, K. T. and Hall, J. L.: In vitro tests of reproductive and developmental toxicity: (1) postimplantation embryos in culture and (2) interspecies in vitro fertilization. Environ. Hlth. Perspec. (In press).

Dixon, R. L. and Hall, J. L.: Reproductive toxicology. In Hayes, A. W. (Ed.): Methods of Toxicology. New York, Raven Press, 1980 (In press).

COMPARATIVE MEDICINE BRANCH

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Summary Statement

The Comparative Medicine Branch (CMB) of the NIEHS 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 laboratories in microbiology, experimental surgery, laboratory animal medicine and mammalian reproduction; maintains glassware and media kitchen serving the Institute; and plans and conducts research appropriate to these laboratory functions.

Administration

Personnel: There are two Veterinarians (one of which is part-time for weekend duty), one Microbiologist, one secretary, two clerks, one Supervisory Animal Husbandman, one Supervisory Biological Laboratory Technician, fifteen Biological Laboratory Technicians, sixteen Biological Aids, seventeen Wage Grades, and thirteen students.

At the present time there are a number of vacancies existing in the Comparative Medicine Branch: two Veterinary Medical Officers, one Veterinary Assistant, one Microbiologist (Virologist), five Biological Aids, two Wage Grades (one of these Permanent Part-Time), four students, and two summer graduates.

Space: There are 30,792 square feet of space in Comparative Medicine. 14,920 square feet of this space is given to animal holding and this is composed of 43 rooms in Buildings 15, 2, 14, 19, 3, the Duke Rodent Breeding Facility and the Duke Vivarium. 13,014 square feet are set aside for support areas, including necropsy, wash area, storage, locker rooms, conference/ training rooms, offices and 772 square feet of the total is for laboratories in veterinary medicine and microbiology. The remaining 2,686 square feet affords vestibules and corridors. Glassware & Media production uses 1,966 square feet of the 30,792.

Programs: The health surveillance program has included the necropsy of clinically ill animals as well as those selected from purchased incoming animals. Microbial results (bacterial, viral serology and parasitology) are summarized and correlated with pathologic findings. Microbial profiles are obtained every 3-6 months from vendors which produce animals for NIEHS on contract. These results are compared with our findings. In addition, microbial profiles are prepared on animals housed at NIEHS to show organisms present in animals within each room or building at NIEHS.

Stay-clean and bioclean rooms have been put in place in animal rooms in Building 15 and studied for preventing cross contamination with bacterial and viral agents. The results to date indicate that the bioclean rooms will not prevent the spread of viruses between animals housed on separate racks within a given room. However, they may be useful in preventing cross contamination from adjoining rooms. Studies are underway to evaluate their effectiveness for preventing cross contamination of viruses in animals housed in separate rooms.

During this time we have received 758 cases (2,497 specimens) and have performed 17,598 tests on these specimens. Viral serology is performed through blanket purchase agreements with Microbiological Associates and with Northrop, Inc. Turnover time continues to be a problem. We still need to develop an in-house virology program.

Environmental Monitoring: We have isolated Salmonella mbandaka from both the meal and pellets which were processed. This has caused the rejection of shipments of feed. A meeting and several discussions have been held with the contractor to establish a quality control program for analyzing the protein supplements for Salmonella prior to being used in diet preparation for NIEHS.

Pest Control: The wild rodent problem experienced a year ago is minimal. A full-time pest control (sanitation) officer is needed to cope with these pest problems both inside and outside our facilities. This will become increasingly important as we occupy the new facility.

Animal Husbandry: During Fiscal Year 1980 (10/1/79 to 10/1/80), over 90,000 animals were purchased for NIEHS. Twenty percent of these were used by the National Toxicology Program. While a number of species have been used, including horses, cattle, sheep, goats, rabbits, guinea pigs and hamsters, over ninety-five percent of those purchased and used were mice and rats.

Over 18,000 animals were maintained on a weekly basis for over 111 research protocols.

Animal Husbandry training was afforded through courses established within the Comparative Medicine Branch. Thirty-two individuals were given training on a continuing basis on basic Animal Care; seven people studied Gnotobiology; six completed the AALAS course and received certification as technicians or technologists; six completed a course in supervision; three in biohazards in the laboratory; and one completed a course on contractural procedures.

Automated Data Processing: A contract was developed through General Services Administration for a systems analyst and time sharing on a computer to initiate an automated data system for CMB. This is to be a detailed analysis of the current manual systems performed by the administrative personnel, animal husbandry and the quality control laboratory. That documentation was submitted and approved May 1980.

Development of System Specifications began immediately thereafter. Equipment needs were determined and estimates on cost submitted. The proposed computer system to replace manual procedures was documented and submitted in August 1980.

Time sharing services were procured in February 1981. All files have been programmed and all screens and error messages are in the process of being programmed.

Research Projects: None.

TOXICOLOGY RESEARCH AND TESTING PROGRAM

TOXICOLOGY RESEARCH AND TESTING PROGRAM

SUMMARY STATEMENT

The Toxicology Research and Testing Program, an integral component of the National Toxicology Program, develops scientific information about potentially toxic and hazardous chemicals which can be used for protecting the health of the American people and for the primary prevention of chemically-induced disease. TRTP concentrates activities in toxicology research, testing and test development/validation efforts, and provides toxicological information needed by research and regulatory agencies. Four specific aims have been identified:

- To expand the toxicological profiles of the chemicals nominated, selected, and being tested.
- To increase, as necessary and as funds permit, the number and rate of chemicals under test.
- To develop and validate a series of tests/protocols more appropriate for regulatory needs.
- To establish and use a coordinated communications network to collect, evaluate, and disseminate toxicological information.

To accomplish these major goals, the program segments are grouped into two categories--toxicologic research and testing, and coordinative management activities.

The research branches embrace Cellular and Genetic Toxicology (Dr. Tennant), Chemical Pathology (Dr. E. E. McConnell), Systemic Toxicology (Dr. J. A. Moore), and Carcinogenesis and Toxicology Evaluation (Dr. J. A. Moore, Acting). The management branches involve Program Operations (Dr. J. F. Douglas), Program Resources (Dr. C. Grieshaber), and Data Management and Analysis (Dr. D. Hoel, this branch is part of the Biometry and Risk Assessment Program). [Each of these discipline areas and their accomplishments are described separately in the sections that follow this overview.]

Individual NTP scientists have been appointed as leaders of major program segments. Each scientist serves as the center for a particular program activity and is responsible for developing (in collaboration with other NTP colleagues) the subprogram objectives and the implementation plan, as well as the coordination and supervision of the program work. Further, the program leaders are responsible for the development and supervision of contracts that extend these activities or that perform in-depth toxicologic characterization of chemicals.

The strategy for test development and validation examines existing and emerging methodologies to identify those which may be adequately sensitive and reproducible. Those offering improvement over older methods will be selected for validation. When basic research findings suggest new areas of toxicology testing, TRTP will undertake the appropriate methods development and validation. Existing methodologies that are being examined for modification include techniques used to detect impaired liver or kidney function and neurobehavioral toxicity; and new areas for methods

development and validation include behavioral teratology, immunotoxicology and short-term tests for presumptive carcinogenic potential. Fertility, reproductive and cardiovascular toxicology test systems are being developed.

Test methods validation signals a two-stage process: (1) Does the procedure(s) yield test results that are reproducible within and between laboratories? (2) Does the test(s) predict for toxic potential in humans? The latter demands that TRTP continue to keep abreast of and examine closely any results from human eidemiologic studies that correlate or contrast with experimental test data. The TRTP approach to testing directs toward developing new and better test methods. This overture does not imply flaws in traditional toxicology and regulatory test requirements, but reflects rapid advancements in testing methodology and expanding boundaries of scientific knowledge. Thus, TRTP plans to validate possible alternatives that may be performed more reliably, yield new toxicologic data, give results relevant to human disease and develop a testing approach that produces equivalent results in a faster, more economical manner. Often, testing results affect regulatory or public health issues, and the TRTP will meld these innovative techniques with "standard" methods to ensure results that are germane and of utility to regulatory and public health needs. When standard methods are used, the TRTP will attempt to incorporate those standards presently advocated by regulatory agencies, such as the life-time rodent bioassay.

PROGRAM OPERATIONS BRANCH

PROGRAM OPERATIONS BRANCH
Summary Statement

The Program Operations Branch has the primary responsibility within the Toxicology Research and Testing Program/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 Section, monitoring of the experiments by the Test Management Section, preparation of the data for publication by the Reports group, verification of the information developed by the Quality Assurance - Good Laboratory Practices Section, and an integration of the NTP facets needed for an efficacious operation by the Planning and Coordination Section.

In addition to its prime functions, members of the Branch participate in other NTP functions as chemical managers, special toxicology advisors, etc.

Collaborative Services

Thirteen laboratories successfully competed and were awarded Basic Ordering Agreements (BOA's) for the possible conduct of bioassays on chemicals in rodents for toxicologic/carcinogenic potential. The pool of qualified laboratories now stands at twenty one as listed in the attached Table 1 and 1A. Eight BOA's expire soon and a readvertisement of the BOA will take place during this fiscal year. The BOA has undergone extensive revision and updating in the interim since the last advertisement.

Forty nine chemicals arranged in 16 packages have been competed among the 21 laboratories in the pool judged qualified to conduct bioassays under the BOA referenced above. Task Order awards to five of the laboratories were completed during FY 1981. A total of $27,327,351 has now been awarded for the conduct of tests on the chemicals shown in Table 2. It is anticipated that task orders for tests on approximately 16 more chemicals will be placed during this fiscal year.

New contracts have also been negotiated for health and safety services, tissue culture studies and studies on 8-methoxy-psoralen. Several project plans have been approved for cell transformation assays, sister chromatid exchange systems, multiple endpoint mutation studies as well as modification of the Salmonella test for chemicals that may be metabolized to mutagens under reductive conditions. The studies in these several project plans are aimed primarily at the development and validation of short-term in vitro methods for detection of the toxicity/carcinogenicity potential of chemicals. Request for proposals have been issued for most of the approved project plans.

Table 1. Laboratories Qualified for Basic Ordering Agreements to Conduct Carcinogenesis Bioassays and Other Toxicologic and Related Studies. The Special Study Capabilities of the Laboratories are Shown in Table 1A

Laboratory	Routes of Administration				
	Dosed Feed	Drinking Water	Gavage	Skin Paint	Inhalation
Battelle Columbus Labs	X	X	X	X	
Battelle Pacific Northwest Labs.	X	X	X	X	X
Bioassay Systems		X	X	X	
EG&G Mason Res. Inst.	X	X	X	X	
Eppley Inst. for Res. In Cancer	X	X	X	X	
Food and Drug Res. Labs	X	X	X	X	
Gulf South Res. Inst.	X	X	X	X	
Hazleton Labs. American	X	X	X	X	X
Illinois Inst. of Technology Res. Inst. (a)	X	X	X	X	X
International Res. and Dev. Corp.	X	X	X	X	X
Inveresk Res. International	X	X	X	X	X
Litton Bionetics	X	X	X	X	X
Microbiological Assoc.	X	X	X	X	
Midwest Res. Inst.	X	X	X	X	X
Oregon State Univ. (a)		X	X	X	
Papanicolaou Cancer Res. Inst. (b)	X	X	X	X	
Raltech Scientific Serv.	X	X	X	X	X
Southern Res. Inst.	X	X	X	X	
Springborn Inst. for Res.	X	X	X	X	
Stanford Res. Inst.	X	X	X	X	
Texas A&M Univ. (a)	X	X	X	X	
Totals	19	21	21	21	8

(a) Prechronic Studies Only
(b) Acute and 14 day repeated-dose only

Table 1A

Laboratory	Special Studies Capabilities				
	Clinical Chemistry	Chemical Disposition	Immuno-Toxicology	Neuro-behavioral Toxicology	Endo-crinology
Battelle Columbus Labs.	Yes	Yes	Yes	Yes	
Battelle Pacific Northwest Labs.	Yes	Yes	Yes	Yes	
Bioassay Systems	Yes				
EG&G Mason Res. Inst.	Yes		Yes	Yes	
Eppley Inst. for Res. In Cancer		Yes			
Food and Drug Res. Labs.	Yes				
Gulf South Res. Inst.	Yes				
Hazleton Labs. American	Yes	Yes	Yes	Yes	Yes
Illinois Inst. of Technology Res. Inst.			Yes		
International Res. and Dev. Corp.	Yes	Yes			
Inveresk Res. International	Yes	Yes		Yes	Yes
Litton Bionetics	Yes	Yes	Yes		
Microbiological Assoc.			Yes		
Midwest Res. Inst.	Yes	Yes			
Oregon State Univ.			Yes		
Papanicolaou Cancer Res. Inst.	Yes				
Raltech Scientific Serv.	Yes				
Southern Res. Inst.	Yes	Yes			
Springborn Inst. for Res.					
Stanford Res. Inst.	Yes	Yes	Yes	Yes	
Texas A&M Univ.	Yes			Yes	
Totals	15	10	9	7	2

Table 2. Chemicals Currently Being Tested Under Basic Ordering Agreements

Battelle Columbus Laboratories

1. p-Chloroaniline (106-47-8)
2. Dimethoxane (828-00-2)
3. Ethylenediamine (107-15-3)
4. Methylolacrylamide (924-42-5)
5. Ochratoxin A (303-47-9)
6. 1-Vinyl-3-cyclohexane dioxide (106-87-6)

EG&G Mason Research Institute

1. 1-Amino-2,4-dibromo-anthraquinone (81-49-2)
2. Chlorpromazine Hydrochloride (69-09-0)
3. Curcuim (458-37-7)
4. 2,4-Diaminophenol Hydrochloride (137-09-7)
5. HC Yellow 4 (52551-67-4)
6. Hexachloroethane (67-72-1)
7. 4-Hydroxyacetanilide (103-90-2)
8. Nitrobenzene (98-95-3)
9. Pentaerythritol tetranitrate (78-11-5)
10. Probenecid (57-66-9)
11. Quercetin (117-39-5)
12. Titanium Ferrocene (1271-19-8)
13. 2,6-Xylidine (87-62-7)

International Research and Development Corporation

1. Azodicarbonamide (123-77-3)
2. β-Cadinene (523-47-7)
3. Carvone (99-49-0)
4. Chloramphenicol (56-75-7)
5. Coumarin (91-65-5)
6. 4,4'-Diamino-2,2-stilbene-disulfonic acid (81-11-8)
7. Diethyl Phthalate (84-66-2)
8. Dihydrocoumarin (119-84-6)
9. Dimethyloldihydroxyethylene Urea (1854-26-8)
10. Isobutyl Nitrite (542-56-3)
11. Isoproterenol Hydrochloride (51-30-9)
12. Mercuric Chloride (7487-94-7)
13. 6-Methylcoumarin (92-48-8)
14. Monochloroacetic acid (79-11-8)
15. Palladium (II) Chloride (7647-10-1)
16. Resorcinol (108-46-3)
17. Toluene (108-88-3)

Microbiological Associates

1. dl-Amphetamine sulfate (60-13-9)
2. Sodium Azide (26628-22-8)
3. Tris (2-chloroethyl) Phosphate (115-96-9)

Southern Research Institute

1. Benzaldehyde (100-52-7)
2. γButyrolactone (96-48-0)
3. C.I. Pigment Red 3 (2425-85-6)
4. C.I. Pigment Red 23 (6471-49-4)
5. Ethylene Glycol (107-21-1)
6. Furan (110-00-9)
7. Furfural (98-01-1)
8. Furfuryl Alcohol (98-00-0)
9. Glycol (polysorbate 80) (9005-65-6)
10. Hexachlorocyclopentadiene (77-47-4)

Good Laboratory Practices Compliance

Toxicological and carcinogenesis animal studies for the Bioassay Program initiated under subcontracts through our Prime Contractor, Tracor Jitco, Inc., and through contracts directly with Toxicology Research and Testing Program/NTP have been done under the Food and Drug Administration's Good Laboratory Practices (GLP) Regulations for Nonclinical Laboratory Studies (Federal Register, December 22, 1978, Part II). Tracor Jitco has established a Quality Assurance Unit to monitor the activities of the Bioassay Subcontracts. A TRTP representative has been selected to work with Tracor Jitco and also to monitor the GLP practices of the TRTP bioassay contractor laboratories. All existing bioassay contracts for animal toxicology carcinogenicity have been modified to include GLP compliance, and site visits are being made to inspect and provide guidance to the laboratory as to how TRTP intends to comply with GLP requirements and will include monitoring of both in-life and data audits.

Full compliance of all contract and subcontract bioassay laboratories conducting carcingenicity studies is scheduled to be in force by September 30, 1981.

Planning and Coordination

During FY 1981 the Planning and Coordination Section has developed procedures for tracking the flow and status of chemicals being tested by the NTP. The procedures identified the major steps in the bioassay testing precess that were to be tracked, documented the material that would be transferred and the personnel that would be responsible for monitoring this process. These procedures were reduced to a list of milestones (Data Elements) that were given to the Computer System group, to develop a Management Information System (MIS) to support bioassay testing. In the interim, the section has developed a procedure to schedule and announce monthly and periodic meetings within the NTP. Further, the section has developed a manual milestone schedule that will be utilized until a MIS has been developed.

Procedures that been developed to schedule and announce monthly and periodic meetings within the NTP. Also procedures have been developed to plan, coordinate and monitor Annual Program Reviews and Quarterly Site Visits.

Currently under development is a procedure to schedule individual NTP bioassay staff functions and monitor the compliance of these functions.

Test Management

The Test Managment Section of the Program Operations Branch is responsible for monitoring toxicological and carcinogenesis studies through contracts directly with NTP. They also help in the monitoring of the performance of the subcontract laboratories that have contracts through the Tracor Jitco, Inc. mechanism. The major emphasis is to insure the scientific and technical quality of the toxicology and bioassay work and to assure the safety of the personnel assigned to test programs. An NTP Senior Staff member is responsible for overall monitoring of the bioassay program and working with a group of NTP Scientific Project Officers assures the scientific integrity and timeliness of the work through regular scheduled site visits and report review. Strong interactions and communications with these individuals and other scientists within NTP (Discipline Monitors and Chemical Managers) and in cooperation with Tracor Jitco representatives and key contract personnel are stressed on a continuing basis.

TRACOR JITCO, INC. - Rockville, Maryland 20852
(NO1-CP-43350)

TITLE: Bioassay Prime Contract

CONTRACTOR'S PROJECT DIRECTOR: Lorne A. Campbell, Ph.D.

PROJECT OFFICER (NCI/NTP): J. Fielding Douglas, Ph.D.
Bioassay Program, NCI/NTP

DATE CONTRACT INITIATED: March 1, 1974

CURRENT ANNUAL LEVEL: $17,280,627

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 NCI/NTP, carry out scientific improvements and cost-saving management methods for the program; (4) purchase chemicals and obtain chemical analysis information on the chemicals to be tested; (5) provide for data submission to the Carcinogenesis Bioassay Data System (CBDS) and assist in the preparation of final reports on the chemicals tested; (6) continue to evaluate, monitor, quality assess and improve pathology services of the program. Hold workshops to improve diagnoses and overall program effort; (7) maintain best effort in performing other tasks as assigned by NCI/NTP.

METHODS EMPLOYED AND MAJOR FINDINGS: Tracor Jitco supports bioassay and toxicological experiments on 152 chemicals in various phases of study. Table 1 lists the chemicals and their status as of 4/1/81. These studies are conducted under subcontracts to 17 facilities and specialized individuals. Table 2 provides the latter information. During the FY 1981, 20 bioassays were completed and technical reports prepared describing the experiments, results and evaluation of the data. The studies were reviewed in public hearings by a peer review subcommittee of the Board of Scientific Counselors. The reports reviewed (all were accepted) were on the following chemicals:

Report	Peer Review Subcommittee Meeting Date
Locust Bean Gum	October 15, 1980
Bisphenol A	"
C. I. Acid Red 14	"
2,6-Dichloro-p-phenylenediamine	"
Di-(2-Ethylhexyl) Phthalate	"
Eugenol	February 18, 1981
C. I. Disperse Yellow	"
Gum Tara	"
D&C Red #9	"

Gum Arabic	February 18, 1981
Vinylidene Chloride	"
C. I. Solvent Yellow #14	"
Agar Agar	"
Guar Gum	"
C. I. Acid Orange	"
11-Aminoundecanoic Acid	"
Stannous Chloride	June 23, 1981
2-Aminobiphenyl	"
Pentachloroethane	"
Allyl isothiocyanate	"

PROPOSED COURSE: It is intended to continue ongoing bioassays of chemicals to completion of the Tracor Jitco Contract, May 31, 1983. The NCI/NTP intends to gradually assume responsibility for the various laboratories beginning in the next fiscal year (FY 1982). All results obtained will be reported in the NTP Carcinogenesis Technical Report Series.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the primary factors basic to the understanding and prevention of cancer is the identification of carcinogenic chemicals in the environment of man. If "environment" is defined to include not only the atmosphere, but also industry, transportation, clothing, housing, occupation, food, drugs, cosmetics, and any other entity with which man comes into contact, the number of suspect chemicals is very large. Thus, the number of chemicals which must be tested is large. Because of the nature of the bioassay, numerous animals must be utilized in testing each chemical over long-time periods. Extensive elements of data are produced which must be recorded accurately and interpreted expertly. This convergence of large-dimension factors results in high costs. To obtain the greatest value in knowledge with minimal expenditures of funds, it is essential that the Program be managed carefully to ensure good science, expert use of manpower and funds, and high quality. A principal goal of the Prime Contractor, therefore, is to achieve the objectives of the NCI/NTP Bioassay Program with high scientific credibility at reasonable costs.

TABLE 1 - BIOASSAY CHEMICALS IN THE PRIME CONTRACT

A. In Chronic Study

Chlorobenzene
o-Dichlorobenzene
p-Dichlorobenzene
Malonaldehyde
Benzene
THPC
THPS
Xylenes
Chlorpheniramine maleate
Orthophenylphenol

Propylene Oxide
Propylene
1,3-Butadiene

Tetrachloroethylene (gavage)
Sodium (2-ethylhexyl) alcohol sulfate
Sodium dodecylsulfate
Ethylene glycol, monoethyl ether
Dodecyl alcohol, ethoxylated
Fluorescein sodium
1,1,1-Trichloroethane
Trichlorfon
Diethanolamine
Gilsonite
Pyridine
Maleic hydrazide, diethanolamine salt
Witch hazel
t-Butyl alcohol
Sodium xylene sulfonate
Castor Oil
Glutaraldehyde
DMBA/TPA
1,2-Epoxyhexadecane

Decabromodiphenyl oxide
Chlorendic acid

Ampicillin Trihydrate
Penicillin V K

Toluene diisocyanate
Diallylphthalate
Ethylene chlorohydrin
Dimethylmorpholinophosphoramidate
Tris(2-ethylhexyl)phosphate
Dimethyl hydrogen phosphite
3-Chloro-2-methylpropene
Dimethylvinyl chloride
Navy fuel JP 5
4-Vinylcyclohexene

p-Rosaniline hydrochloride salt
Diglycidylresorcinol ether
Monuron
1,2-Dichloropropane
Chlorodibromomethane
Bromodichloromethane
8-Hydroxyquinoline
n-Butyl chloride
Chlorinated trisodium phosphate

Trichloroethylene
Isophorone

Ephedrine sulfate
Phenylephrine HCl
Erythromycin stearate
Oxytetracycline

Allyl isovalerate
Ethylacrylate
HC Blue 1
HC Blue 2
HC Red 3
Disperse Blue 1
Acid Orange 3
Chlorowax 40
Chlorowax 500C
Rhodamine 6G

B. In Prechronic Study

N-Phenyl-beta-Naphthylamine
2,4-Dichlorophenol
Sodium fluoride
Pentachlorophenol (pure)
Pentachlorophenol (technical)

Boric Acid
Pentachloronitrobenzene
Phenylbutazone

Benzyl Alcohol

TABLE 1 - BIOASSAY CHEMICALS IN THE PRIME CONTRACT (continued)

Pentachlorophenol (Dowicide EC-7)
Pentachlorophenol (DP-2)
Rotenone

1,2-Epoxybutane
Methyl Methacrylate
Ethylene Oxide
Chlorobenzalmalononitrile and
Hexamethyldisilizane (CS2)
Tetrachloroethylene (Inhalation)
Methylene Chloride (Inhalation)
Ethyl Bromide
Ethyl Chloride
Allyl Glycidyl Ether
alpha-Chloroacetophenone
Epinephrine
Formaldehyde

Hydroquinone
Catechol
p-Quinone

Phenolphthalein

Coconut Oil, DEA
Lauric acid, DEA
Oleic acid, DEA
Chloramine
Hematoxylin
p-Nitrophenol
2-Butanone peroxide

Diesel Fuel Marine
Dimethyl methyl phosphonate
Bromoform
Iodinated Glycerol

Succinic Anhydride
alpha-Methylbenzyl Alcohol
d-Limonene
Methyl Carbamate

Vinyl Toluene
Tetranitromethane

Bromobenzene
2,3-Dibromo-1-propanol
2,2-Bis(bromomethyl)-1,3-propanediol
Glycidol

Nalidixic Acid
Tetracycline HCl
Methyldopa
2-Amino-4-Nitrophenol
2-Amino-5-Nitrophenol
Hexylresorcinol
Nitrofurazone
Mercaptobenzothiazole

Pentachloroanisole
Nitrofurantoin
Roxarsone
Dichlorvos
Wollastonite

Benzofuran
N,N-Dimethylaniline

Dephenhydramine Hydrochloride
8-Methoxypsoralen
Furosemide
Hydrochlorothiazide
5-Hydroxytryptophan

C. In the Report Preparation Stage

Ascorbic acid
1,1,1,2-Tetrachloroethane
Methylene chloride (gavage)
Melamine
Bis(2-chloroisopropyl) ether
Mannitol

Ziram
Benzyl acetate
Geranyl acetate
Zearalenone
Propyl gallate
4,4'-Methylenedianiline

TABLE 2 - SUBCONTRACTORS TO THE PRIME CONTRACT

Battelle-Columbus Laboratories
Battelle-Pacific Northwest Laboratories
Bioassay Systems, Inc.
Bio-Serv, Inc.
EG&G Mason Research Institute
Gulf South Research Institute
Hazleton Laboratories America, Inc.
Litton Bionetics, Inc.
Microbiological Associates
Midwest Research Institute (chemistry)
Midwest Research Institute (inhalation)
NuChem Co., Inc.
Papanicolaou Cancer Research Institute
Physiological Research Laboratories
Southern Research Institute
Springborn Institute for Bioresearch
SRI International

Title: Pulmonary Tumor Induction in Strain A Mice (NO-1-CP-95608) and Validation of the Lung Tumor Assay. (NO-1-CP-90212)

UNIVERSITY OF CALIFORNIA, SAN DIEGO - NO-1-CP-95608

Principal Investigator: Dr. Michael B. Shimkin
NTP Project Officer: Dr. William V. Hartwell
Date Initiated: October 1, 1979 Current Annual Funding Level: $171,214

OAK RIDGE NATIONAL LABORATORIES, OAK RIDGE, TENNESSEE - NO-1-CP-90212

Principal Investigator: Dr. H. P. Witschi
NTP Project Officer: Dr. William V. Hartwell
Date Initiated: October 1, 1979 Current Annual Funding Level: $210,000

Objective: To evaluate the pulmonary tumor induction as a screening technique for prioritizing select chemicals for testing by the Carcinogenesis Bioassay and to validate the evaluation. Induction of pulmonary adenomas by administration of chemicals to susceptible strains of mice such as the A strain has been used to study chemically induced cancer for a number of years. The incidence of lung tumors in susceptible strains increase with age of the animal, and incidences greater than 50% are not uncommon among old mice. When young mice are exposed to known carcinogens the incidence of pulmonary tumors is significantly higher than the vehicle or untreated controls within 6-7 months. Chemicals tested at the University of California, San Diego and at Oak Ridge National laboratories were furnished under code from the repository of chemicals previously tested by the NCI two year bioassay. Tumor induction was to be studied by the University of California, San Diego, and the results were to be validated with select chemicals at Oak Ridge National Laboratories.

The test consists of two phases. In the first phase, groups of 6-8 week old mice are injected peritoneally with different levels of test chemical in tricaprolin or saline, 3 x per week on alternate days for two weeks followed by a two week observation period. Solution of high dose for chronic study is based on survival and absence of life threatening lesions. In the chronic phase groups of 20-30 6-8 week old mice are injected peritoneally with the high dose determined by the first phase, ½ the high dose and ¼ the high dose in the vehicle use in the first phase. Dosing is performed 3 times per week for eight weeks followed by 16-18 weeks observation after which animals are necropsied and the lungs are removed and fixed for scoring. A matched control, vehicle controls and a positive control group are included with each group of chemicals tested.

Major Findings: Testing at the University of California, San Diego is nearing completion and data is being entered into the computer for further analysis. At Oak Ridge National Laboratories 13 chemicals have been tested and 13 are on test. Upon receipt of the final project report from the University of California, San Diego, that code will be broken and the results compared with other available data.

Significance to Biomedical Research and the National Toxicology Program: The National Toxicology Program was established to centralize and strengthen DHHS activities in toxicology research and testing and to provide necessary toxicological information needed by research and regulatory agencies. Since over 50 percent of the chemicals tested by the NCI Bioassay did not induce significant incidences of tumors in rats and mice, use of a short term *in vivo* assay for prioritizing chronic testing of candidate chemicals would enhance testing of likely carcinogens.

Proposed Course: Because of the small number of candidates tested by this model to date, the Strain A mouse should be derived in a pathogen free state to permit its use in barrier facilities in conjunction with chronic bioassays currently in progress.

PROGRAM RESOURCES BRANCH

Annual Report of the Program Resources Branch
National Toxicology Program

The Program Resources Branch is responsible for providing those elements which are essential to the performance of toxicity and carcinogenicity studies by the National Toxicology Program. The Branch is responsible for procuring and analyzing chemical compounds and supplying disease-free animals. These elements serve as the "test system" for in vivo studies. In order to protect the laboratory worker and his/her environment a Safety Section is responsible for providing health and safety monitoring and facility procedures which protect the individual. Additionally, the Branch interacts with the scientific public by providing information of a technical nature to these individuals. Each one of these resources is provided by a significant in-house effort and supplemented by resources contracts.

Analytical chemistry services were provided to the Bioassay Program, the National Center for Toxicological Research and the National Institutes of Occupational Safety and Health. Over 70 chemical compounds were procurred and analyzed for use in the varied NTP programs.

Approximately 400 compounds have been selected for mutagenicity testing. These compounds were analyzed and placed in our repository for use in FY '81. Additionally 368 compounds which have been tested for toxicity are inventoried in our chemical repository for reference use by the NTP or other interested parties.

Over 3000 total disease free, genetically defined F344 rats and B6C3F1 mice were produced weekly during FY '81. Each animal was used as part of the "test system" described previously.

The Program Resources Branch through the Technical Information Section (TIS), is responsible for dissemination of all technical information for the Toxicology Program. This responsibility also implies the gathering of information from the various program areas so that it is resident in one centralized location and maintained so that it is easily accesible to any qualified user. The TIS updates, maintains and distributes the quarterly report "Chemical on Standrad Protocol in the Carcinogenesis Bioassay Program" which is distributed to 500 recipients who need to be appraised of the status of compounds during bioassay.

The chemical managers of the NTP rely on TIS for information pertaining to their compounds. Contract mechanisms are used to obtain indepth searches on compounds as well as current awareness updates. In FY '81 40 of these contracted searches were delivered to the chemical managers. In addition, numerous ad hoc computer bibliographic searches were done by TIS personnel.

This year the responsibility for producing and disseminating the NTP Technical Bulletin was assumed by TIS personnel. In April, Issue 4 was completed and distributed to approximately 4,000 subscribers.

In FY 81, TIS became the clearinghouse for all publications, seeing to the printing and distribution of drafts for the peer reviews of technical reports in February and May, providing the handouts for the review meetings, and

assuming responsibility for production and distribution of all technical reports as well as distributing the NTP Annual Plan and the Annual Reports on Carcinogens.

TITLE: Rodent Production Contracts

PROJECT OFFICER: Dr. Gary A. Boorman

CO-PROJECT OFFICER: Mr. Clarence Reeder/NCI

INITIATION DATE: 9/30/79

EXPIRATION DATE: 9/29/84

CHARLES RIVER BREEDING LABORATORIES, PORTAGE NO1-CP-95641
CONTRACTOR'S PROJECT DIRECTOR: Sumner J. Foster
FUNDING LEVEL: $1,374,767

CHARLES RIVER BREEDING LABORATORIES, STONERIDGE, NY NO1-CP-95617
CONTRACTOR'S PROJECT DIRECTOR: Sumner J. Foster
FUNDING LEVEL: $1,419,383

HARLAN INDUSTRIES, INCORPORATED NO1-CP-95642
CONTRACTOR'S PROJECT DIRECTOR: Hal P. Harlan
FUNDING LEVEL: $1,376,360

SIMONSEN LABORATORIES, INCORPORATED, GILROY, CA NO1-CP-95643
CONTRACTOR'S PROJECT DIRECTOR: Harry Simonsen
FUNDING LEVEL: $1,258,405

PROJECT DESCRIPTION

OBJECTIVES: To produce disease-free genetically defined F344 and B6C3F1 hybrid mice for the Bioassay Program.

METHODS EMPLOYED: Genetically defined pedigree stock is obtained from the NIH Repository. Cesarean derived offspring are maintained in germ-free isolators, given defined bacterial flora and maintained in isolators as inbred foundation. These animals are used to supply production colonies. 300 of each sex and species (Total 1200 animals) are produced weekly per each laboratory, weaned 1 week prior to shipment and shipped at 5-6 weeks of age to Bioassay contracted labs. Pedigreed stock and breeders are replaced as scheduled.

MAJOR FINDINGS AND PROPOSED COURSE: Rats and mice are the main test systems for the Bioassay Program. Over the past year no chemical starts have been delayed more than two weeks because of animal shortages. The animals produced continue to be free of infectious disease. Mouse shipments were halted briefly at Harlan because Mouse Hepatitis Virus was found in one of the isolators.

Simonsen has Sendai Virus infection in the animal production rooms and has therefore maintained the inbred lines but has not gone into production. They have proposed and we have accepted their offer to install at their expense an isolator room for rodent production. The room size limits the production to 200 per sex per species per week.

An agreement was reached with the Division of Cancer Treatment at FCRC to produce rodents for the Bioassay Program. They are currently providing the Program with an average of 300 B6C3F1 Mice per sex per week. Very shortly this will be expanded to include the same number of Fischer 344 rats.

Since rodent production will be adequate with the DCT/FCRC agreement, the Harlan contract will be terminated on 30 April, 1981 due to noncompliance to contractual requirements.

Production Figures:

Charles River, Portage	300 B6C3F1 Mice per sex per week 300 Fischer 344 Rats per sex per week
Charles River, Kingston	300 B6C3F1 Mice per sex per week 300 Fischer 344 Rats per sex per week
Harlan	Average of 225 per species per sex per week

Average expenses incurred per month:

Charles River, Portage	$24,000
Charles River, Kingston	$21,000
Harlan	$24,000

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE NATIONAL TOXICOLOGY PROGRAM: The NTP has a mandate to determine the toxic and carcinogenic potential of chemical compounds found in the environment. Disease-free, genetically defined F344 and B6C3F1 hybrid mice are the essential test elements of the Bioassay Program conducted by the NTP to detect toxic and carcinogenic effects.

TITLE: Diagnostic Laboratories

PROJECT OFFICER: Dr. Gary A. Boorman

CO-PROJECT OFFICER: Mr. Clarence Reeder

INITIATION DATE: 9/30/79

EXPIRATION DATE: 9/29/84

RODENT DISEASE DIAGNOSTIC LABORATORY NO1-CP-95616
BOARD OF TRUSTEES AT UNIVERSITY OF ALABAMA
FOR UNIVERSITY OF ALABAMA IN BIRMINGHAM
UNIVERSITY STATE
BIRMINGHAM, ALABAMA 35294
CONTRACTOR'S PROJECT DIRECTOR: Russel Lindsay
FUNDING LEVEL: $688,851

PROJECT DESCRIPTION

OBJECTIVES: To provide rodent disease diagnostic laboratory support for monitoring the health status of animals used by the Bioassay Program.

METHODS EMPLOYED: A battery of test procedures are performed on serum samples from "sentinel" animals in each animal room in contractor facilities. Immunological procedures are utilized to screen for micoplasma, pinworm, arthropod and bacterial pathogens and viral serology. The results of this diagnostic testing are reported to the Project Officer. Additional investigation such as diagnosis of disease outbreaks in NCI stocks are similary undertaken as needed and in accordance with the general goal of eliminating all spontaneous diseases as sources of variability in these colonies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is essential to the conclusions derived from testing that the bioassay be performed using disease-free species.

Battelle Memorial Institute, Columbus, OH	CP9-5659
Enviro Control, Rockville, MD	CP9-5662
Franklin Institute Research Labs, Philadelphia, PA	CP9-5658
JRB Associates, Inc., McLean, VA	CP9-5660
Mason Research Institute/EG&G Mason, Rockville, MD	CP9-5657
Tracor Jitco, Inc., Rockville, MD	CP9-5621
VSE Corporation, Alexandria, VA	CP9-5661

TITLE: Information Resources Basic Ordering Agreement

CONTRACTORS PROJECT DIRECTOR: (Battelle - R. Weiner; Enviro - M. Palmer; Franklin - G. Campbell; JRB - D. Heldreth; Mason - M. O'Flaherty; Tracor Jitco - R. Huffman; VSE - W. Miller)

PROJECT OFFICER (NCI/NTP): Joan W. Chase, M.S. Acting Head, Technical Information Section, Program Resources Branch

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $200,000

OBJECTIVES: To supply information and systems services quickly and inexpensively to NTP staff.

PROJECT DESCRIPTION

METHODS EMPLOYED: This Basic Ordering Agreement has been used to obtain information science and systems services on an as needed basis. Requests for services are submitted by the staff. These requests are then competed as tasks among the contractors competent in information services (enviro, Franklin, JRB and Tracor Jitco) or systems services (Battelle, JRB, Mason, VSE.) Tasks in FY '80 included specialised literature searches on compounds in the program, generation of safety reports so that testing laboratories have instructions on how to handle the compounds, specialized keyboarding help for input of NTP data, acquisition of reprints and copies of journal articles for chemical managers who use the information in their experimental design.

MAJOR FINDINGS AND PROPOSED COURSE: The contracts have served the purpose for which they were designed; quick response at reasonable cost. In April another RFP for continuation of information sciences services was released and multiple awards made to the contractors who responded and fell into the competetive range. The systems portions of the RFP was dropped since under the NTP reorganization, that area no longer falls in Program Resources.

SIGNIFICANCE....ETC.

The project as stated before, has allowed Program staff to obtain necessary information for experimental design and safety. It also provided systems services for maintenance of computer data bases.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION- Oak Ridge, Tennessee
(CP-2-0202)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: John Wassom

PROJECT OFFICER (NCI/NTP): Joan W. Chase, M.S. Acting Head, Technical Information Section, Program Resources Branch.

DATE CONTRACT INITIATED: June 30, 1980

CURRENT ANNUAL LEVEL: $60,500

PROJECT DESCRIPTION

OBJECTIVES: To maintain the Environmental Mutagen Information Center (EMIC) as a unique resource for the acquisition and maintaenance of mutagenesis information. This information maintained not only at Oak Ridge in the files of EMIC but records are available on-line through the Department of Energy's RECOM System and and the National Library of Medicine's TOXLINE. Thus, they are available to anyone who has access to these systems.

METHODS EMPLOYED: NCI/NTP acts merely as a funding agency for EMIC. The Center is administered through the NIEHS Project Officer, Dr. H. Malling.

MAJOR FINDINGS AND PROPOSED COURSE: EMIC has been continuing its information services and NCI/NTP has assumed the responsibility of funding it for an additional $121,000 for FY '81.

SIGNIFICANCE: The NTP has a large mutagenesis testing component. EMIC serves as an information base for this part of the Program.

RADIAN CORPORATION - AUSTIN, TEXAS
(NO1-CP-95649)

TITLE: Chemical Repository for the National Toxicology Program

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $74,350

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for the chemicals which are studied by the National Toxicology Program. All available physical-chemical and toxicological information is provided on all chemicals.

METHODS EMPLOYED: The contractor receives chemicals which have been or will be tested by the National Toxicology Program. Information is compiled for safety and handling documents as well as complete data sheets on physical-chemical properties, structure, name, toxicology and miscellaneous information. The materials are divided into appropriate aliquots, indexed, cross referenced and inventoried into a computerized system. The chemicals are available to both NTP and non-NTP investigators who may have a need for a sample of a compound which has been tested by the NTP.

MAJOR FINDINGS AND PROPOSED COURSE: The current inventory at the chemical repository is 368 chemicals. All chemicals are stored in the Contractor's Hazardous Materials Laboratory. Catalog file data (chemical properties etc.) are compiled as compounds are placed in inventory status. Work has been completed on a computerized inventory and data handling system. Approximately 200 aliquots of chemicals have been shipped in FY 80. Also, approximately 100 aliquots of chemicals were transfered to the NIEHS Chemical Repository for Mutagenicity Screening.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of enviromental chemicals. The objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The maintance of a chemical repository to serve as a central archive for the storage and distributation of chemicals studied by the NTP is an essential part in maintaining the quality of the Program.

MIDWEST RESEARCH INSTITUTE - KANSAS CITY, MISSOURI
(NO1-CP-95615)

TITLE: Chemical Servies Support for the National Toxicology Program

CONTRACTOR's PROJECT DIRECTOR: E. J. Woodhouse, Ph.D.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: September 30, 1979

CURRENT ANNUAL LEVEL: $1,689,427

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to provide chemical procurement, analysis, storage, repackaging, and distribution services in support of the activities of the National Toxicology Program. The contractor serves as an analytical resource for the NTP performing analysis of chemicals for identity and purity assay and stability; formulation of protocols for chemical mixes; analysis of feed samples for toxic components and analysis of dose-feed samples. Special tasks also include isolation and identification of impurities, tissue residue analyses and other associated analytical problems.

METHODS EMPLOYED: The contractor procures and receives chemicals which are to be tested by various Programs in the NTP including both contract and in-house laboratories. Data is generated on the identity and purity of each test material. In addition stability assays are performed to determine the long term stability of both the bulk chemical and chemical-vehicle mixtures. Also, methods are developed for the assay of the test material in the vehicle used in the toxicity test. Other assays such as tissue residue analysis and identification of minor impurities are performed as required.

MAJOR FINDINGS AND PROPOSED COURSE: For FY 80, 51 chemicals were procured or synthesized and analyzed for carcinogenesis bioassay testing. In addition, 12 chemicals were procured and analyzed for teratology studies. Additional analytical services were provided to the National Center for Toxicological Research in support of 8 chemicals being tested for the National Toxicology Program. Work was also completed on the tissue residue analysis for 6 chemicals being studied by various members of the intramural staff of the NTP. Future plans include continued support of the above mentioned activities as well as support of additional teratology studies by NIOSH and a NTP fertility testing contract to be awarded in FY82.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of environmental chemicals. The objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The procurement and analysis of chemicals is one of the essential steps in the success of bioassays. Without this activity, no substantive animal or in vitro testing could occur. The precise definition of the chemical nature of compounds is one of the cornerstones in an effort to increase the accuracy and reliability of data obtained in toxicological research.

PUBLICATIONS

Murrill, E.A., Woodhouse, E.J., Olin, S.S., Jameson, C.W.: Carcinogenesis Testing and Analytical Chemistry. Analytical Chemistry 52: 1188A, 1980

UNIVERSITY OF NEBRASKA MEDICAL CENTER
EPPLEY INSTITUTE FOR RESEARCH IN CANCER, OMAHA, NEBRASKA
(NO1-CP-05629)

TITLE: Carcinogenesis, Chemistry and Biological Actions of Hydrazines

CONTRACTOR'S PROJECT DIRECTOR: Bela Toth, D.V.M.

PROJECT OFFICER (NTP): C. W. Jameson, Ph.D., Program Leader/Chemistry, NTP

DATE CONTRACT INITIATED: January 16, 1980

CURRENT ANNUAL LEVEL: $185,160

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to study the toxicologic and carcinogenic properties of environmentally important hydrazines.

METHODS EMPLOYED: Eppley Swiss Mice or Syrian Golden Hamsters were exposed to the test chemicals for their lifetime by various routes of administration (e.g. drinking water, iv injection, gavage, etc). Most animals are dead and the few remaining ones will be terminated by the end of June, 1981. From the tissues of experimental animals, histology slides have been or will be prepared and evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: A very high percentage (70%) of the hydrazines studied in life long experiments have been found to be carcinogenic in laboratory animals. This contract will be terminated on June 15, 1981.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been demonstrated that in addition to the well-known nitrosoamines and azo compounds, the hydrazines represent a new group of N-N bond containing compounds of which a large majority are carcinogenic. Careful consideration should be given to the potential health hazard posed by these hydrazines to the human population.

PUBLICATIONS

Toth, B. and Nagel, D.: N-Ethyl-N-formylhydrazine tumorigenesis in mice. Carcinogenesis, 1: 61-65, 1980.

Toth, B.: Carcinogenesis by gyromitrin of Gyromitra esculenta. Federation Proc., 39: 884, 1980.

Toth, B., Nagel, D. and Ross, A.: Occurrence and the carcinogenic action of 4-(hydroxymethl)benzene diazonium ion (4-HMBD). Proc. Am. Assoc. Cancer Res., 21:73, 1980.

Toth, B. and Patil, K.: The tumorigenic effect of low dose levels of N-methyl-N-formylhydrazine in mice. Neoplasma, 27: 25-31, 1980.

Toth, B.: Actual new cancer-causing hydrazines, hydrazides and hydrazones. J. Cancer Res., Clin. Oncol., 97: 97-108, 1980.

Toth, B. and Patil, K.: Carcinogenesis by a single dose of N-Methyl-N-formylhydrazine. J. Tox. Env. Health, 6: 577-584, 1980.

Toth, B.: Carcinogenesis by mushroom hydrazines. Additional investigations with naturally occurring mycotoxins. In "Biology of the Cancer Cell". Proc. V. Meeting of Europ. Assoc. Cancer Res., Vienna, Austria, 45-50, 1980.

Toth, B., Nagel, D. and Patil, K.: Tumorigenic action of N-n-butyl-N-formylhydrazine in mice. Carcinogenesis 1: 585-593, 1980.

Malick, L. and Toth, B.: Histochemical and cytochemical studies of angiosarcomas in mouse liver. J. Histochem. Cytochem. (submitted for publication).

Toth, B.: Nicotinic acid hydrazide carcinogenesis in mice. Oncology (in press) 1980.

Toth, B., Nagel, D. and Patil, K.: Tumorigenesis by N-n-propyl-N-formylhydrazine in mice. Brit. J. Cancer (in press).

Toth, B. and Nagel, D.: Carcinogenesis by mycotoxins of two edible mushrooms. Proc. Intern. Acad. Pathol., (in press).

Toth, B., Smith, J. and Patil, K.: Cancer induction with acetaldehyde methylformylhydrazone of the false morel mushroom. J. Nat. Cancer Insti., (submitted for publication).

Toth, B. and Patil, K.: Gyromitrin as a tumor inducer. Neoplasma (submitted for publication).

Toth, B.: Influence of chain length on N-alkyl-N-formylhydrazine carcinogenesis. Federation Proc., (submitted for publication).

Toth, B. and Nagel, D.: 1,2-di-n-Butylhydrazine dihydrochloride carcinogenesis in mice. Experientia (submitted for publication).

Toth, B. and Jae, H.S.: Carcinogenesis and chemistry of mycotoxins and related compounds of Agaricus bisporus. Proc. Am. Assoc. Cancer Res. (submitted for publication).

Kuszynski, C., Langenbach, R., Malick, L., Tompa, A. and Toth, B.: Liver cell-mediated mutagenesis of V-79 cells by hydrazine and related compounds. Environmental Mutagenesis (submitted for publication).

Toth, B.: Hydrazines and related compounds in colonic carcinogenesis. Falk Symposium, MTP Press, Lancaster, 1981 (submitted for publication).

Toth, B., Ross, A., Patil, K. and Jae, H.S.: Carcinogenesis and chemistry of 4-(hydroxymethyl)benzenediazonium ion(tetrafluoroborate) of Agaricus bisporus. Cancer Res. (submitted for publication).

Ross, A., Nagel, D. and Jae, H.S.: The identification of aromatic diazonium ions in mushroom extracts. Proc. Am. Assoc. Cancer Res., (submitted for publication).

RADIAN CORPORATION - AUSTIN, TEXAS
(NO1-ES-8-2144

TITLE: Chemical Repository for Mutagenicity Screening

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D.

PROJECT OFFICER (NTP): Douglas B. Walters, Ph.D., Program Leader/Chemical Health and Safety, NTP

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $370,000

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for 3000-4000 compounds which are to be screened for mutagenicity in the National Toxicology Program. Available physical - chemical and toxicological information is provided on all compounds and chemical analysis is performed when required.

METHODS EMPLOYED: The contractor receives chemicals which are to be tested for mutagenicity by laboratories under contract. Information is compiled for safety and handling documents as well as data sheets on physical - chemical properties, structure, name, toxicology and miscellaneous information. The materials are coded for blind testing, divided into appropriate aliquots, indexed, cross referenced and inventoried into a computerized system. The compounds are sent by a safe, appropriate route to the testing laboratory at a rate of about 30 samples per month including controls. An estimated 10% of test compounds are analyzed for trace chemical impurities. Three compounds have been synthesized for special NTP needs.

MAJOR FINDINGS AND PROPOSED COURSE: Approximately 396 test compounds and 25 controls have been selected for FY 81 testing. Currently, inventory at the repository is 520 chemicals. All chemicals are stored in the Contractors Hazardous Materials Laboratory. Catalog file data (chemical properties, etc.) are compiled as compounds are placed in inventory status. Work has been completed on implementing a computerized inventory and data handling system. An addition to the Hazardous Materials Laboratory is under construction and completion is anticipated this fiscal year. Satisfactory survivability tests have been completed and approximately 500 aliquots will be shipped in FY 81. Approximately 50 chemicals will be subjected to chemical analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a comprehensive testing system for mutagenesis (as well as other toxicity testing) requires a repository which can be computerized for effectiveness and efficiency and which is designed around a specially designed containment laboratory for handling hazardous materials. The laboratory also must provide for routine chemical assay as well as sophisticated, complete, chemical trace impurity analysis. These requirements are necessary to support *in vitro* and *in vivo* testing.

PUBLICATIONS

Moore, J.A., Huff, J.E., Hart, L., Walters, D.B.: Overview of the National Toxicology Program in Environmental Health Chemistry: The Chemistry of Environmental Agents as Potential Human Hazards. Chapter 25, Ann Arbor Science, Ann Arbor, Michigan, 1981.

Walters, D.B., Keith, L.H. and Harless, J.: Chemical Selection and Handling Aspects of the National Toxicology Program, in Environmental Health Chemistry: The Chemistry of Environmental Agents as Potential Human Hazards. Chapter 26, Ann Arbor Science, Ann Arbor, Michigan, 1981.

Keith, L.H., Harless, J.M., Ward, J.T. and Walters, D.B.: Analysis and Storage of Hazardous Environmental Chemicals for Toxicological Testing, in Environmental Health Chemistry: The Chemistry of Environmental Agents as Potential Human Hazards, Chapter 27, Ann Arbor Science, Ann Arbor, Michigan, 1981.

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, ACS Symposium series, Washington, D.C., 1981 (in press).

IIT RESEARCH INSTITUTE - CHICAGO, ILLINOIS 60616
NO1-ES-1-5003

TITLE: Synthesis of Selected Tetrachlorodibenzo-p-dioxins and Related Compounds as Analytical Standards

CONTRACTOR's PROJECT DIRECTOR: John T. Uchic

PROJECT OFFICER (NIEHS): J.D. McKinney, Ph.D., Chief, LEC
D.B. Walters, Ph.D., Program Leader/Chemical Health Safety, NTP

DATE CONTRACT INITIATED: March 1, 1981

CURRENT ANNUAL LEVEL (1 year): $126,738

PROJECT DESCRIPTION

OBJECTIVES: To obtain sufficient quantities of selected compounds in sufficient purity for use as analytical standards. The unequivocal determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has not been reported to date and primarily due to lack of valid, accurate, and reliable analytical methods based on proper analytical standards. This study will provide the necessary analytical standards to support such analytical method developments.

METHODS EMPLOYED: A synthetic method is required for each sample which will produce the least number of isomers; thereby simplifying structure assignment (by ^{13}C and ^{1}H NMR as well as x-ray crystallography) problems. Various separation techniques such as HPLC will be employed to separate and purify each isomer.

MAJOR FINDINGS AND PROPOSED COURSE: To prepare 0.1 to 1 gram quantities each of 12 halogenated dibenzo-p-dioxin congeners and related compounds which represent potential interferences in the chemical analysis of 2,3,7,8-TCDD. These compounds must have a purity 98% and sufficient chemical characterization data will be submitted for each compound to permit unequivocal structure assignment.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM AOF THE INSTITUTE: The unequivocal determination of 2,3,7,8,-TCDD, one of the most toxic substances known to mankind and with potential interference from numerous other isomers, is dependent upon obtaining pure uncontaminated standards of these other isomers as reference materials. TCDD's are possible widespread contaminants of such herbicides as 2,4,5-T and 2,4-D. This study will provide the basis to individually analyze these TCDD isomers.

A.D. LITTLE, INC, - CAMBRIDGE, MASSACHUSETTS
(NO1-CP-05673

TITLE: Health and Safety Services Support for the Carcinogenesis Testing Program

CONTRACTOR'S PROJECT DIRECTOR: R. Scott Stricoff

PROJECT OFFICER (NTP): Douglas B. Walters, Ph.D., Program Leader/Chemical Health and Safety, NTP

DATE CONTRACT INITIATED: September 30, 1980

CURRENT ANNUAL LEVEL: $305,453

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to assist the National Toxicology Program in the evaluation of health and safety practices of the NTP and its contract laboratories. Assistance provided includes: basic industrial hygiene as well as specialized experience in analytical chemistry, engineering control, personal protective equipment evaluation, human factors evaluation and statistical design of sampling strategies.

METHODS EMPLOYED: The Contractor furnishes services, qualified personnel, material, equipment, and facilities as needed to evaluate, survey and assist the NTP in the following areas:

Design, implementation, and qualitative and quantitative evaluation of industrial hygiene and pollution monitoring programs at designated facilities to examine the spread of NTP test materials.

Development and evaluation of alternate work practices or engineering controls in alleviating potentially hazardous situations encountered in NTP facilities.

Inspection of laboratory facilities for qualitative evaluation of the health and safety program.

MAJOR FINDINGS AND PROPOSED COURSE: Coordination and planning meetings have occured and include the health and safty evaluation of: NTP contract laboratories in the Cellular and Genetic Toxicology program and all other NTP testing efforts; procedures used for disposal by incineration of surplus NTP chemicals; medical monitoring programs applicable to NTP Carcinogenesis Bioassay laboratories; the health and safety programs; procedures and facilities of current carcinogenesis bioassay laboratories via participation and assistance in the routine monitoring and inspection program: practices and equipment used for personal protection.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The National Toxicology Program has a national mandate to determine the toxicologic potential of environmental chemicals. the objective is primarily attained by the bioassay of various chemicals in both long term animal studies and short-term tests. The maintance of effective Health and Safety Program is an essential part in maintaining the quality of the Program.

DEPARTMENT OF ENERGY - OAK RIDGE NATIONAL LABORATORY
(222 Y01-ES-10004)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: John Wassom

PROJECT OFFICER (NIEHS): John Moore, D.V.M.

DATE INTERAGENCY INITIATED: FY 1971

CURRENT ANNUAL LEVEL: $360,000

PROJECT DESCRIPTION

Chemical Mutagenesis Literature- Supported by the NTP, the Environmental Mutagen Information Center (EMIC) collects, organizes, and disseminates primarily published information on chemicals tested for mutagenicity. Located since inception in 1969 at the Oak Ridge National Laboratory, the EMIC computerized data file contains 36,555 (July 1981) 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 36,555 documents equals 10,000.

DEPARTMENT OF ENERGY - OAK RIDGE NATIONAL LABORATORY
(222 Y01-ES-50010)

TITLE: Environmental Teratology Information Center

CONTRACTOR'S PROJECT DIRECTOR: John Wassom

PROJECT OFFICER (NIEHS): John Moore, D.V.M.

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. Through July 1981, the ETIC data file contains 23,199 records, the majority of which are available online from TOXLINE and from RECON. The number of unique chemicals identified from these 23,199 documents equals 5,700.

ETIC, located at NIEHS, has established a microform document library containing copies of 21,000/23,199 (91%) 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.

CARCINOGENIC AND TOXICOLOGIC EVALUATION BRANCH

CARCINOGENIC AND TOXICOLOGIC EVALUATION BRANCH

SUMMARY STATEMENT

The Carcinogenic and Toxicological Evaluation Branch (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 within other scientific staff in the National Toxicology Program involved in test development and validation and test protocol preparation; and (3) monitors testing programs to assure the quality and validity of the toxicity studies.

This is the first year in existence of this branch and the highest priority during this year was given to 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 49 chemicals is being studied under NTP Basic Ordering Agreement with EGRG Mason Research Institute, International Research Development Corporation, Battelle Columbus Laboratories, Microbiological Associates and Southern Research Institute. These studies are at prechronic phase. The branch is managing the chemical toxicity study of 121 chemicals (including chemicals studied in the Tracor-Jitco prime contract). The branch has developed protocols for 14 chemicals for which the contracts are planned to be awarded this fiscal year. The final drafts on 15 technical reports from TJ contract were prepared for studies which have been completed.

- Investigation of the biological, pharmacological and toxicological consequences of the interaction of UV light with psoralen compounds administered orally and to evaluate the ability of 8-MOP to induce cancer in the absence of UV light. Although 8-MOP is the most important psoralen in general chemical use, information on related compounds is sought for comparative purposes. This study is being conducted through five different contracts (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 (Dr. Mennear).

- To determine if there is increased sensitivity of laboratory animals to potential carcinogenic and toxicologic effects of selected chemicals exposed at various levels during their in utero development plus post-natal life of two years as compared to the animals exposed only during post-weaning time of two years. This study is performed through a contract with Battelle Columbus Laboratories. Phenytoin, ethylenethiourea and Firemaster FF-1 (PBB's) are being studied under this contract. At present, these studies are at prechronic phase (Dr. Chhabra).

Intramural Research: The following are the in-house research projects which were initiated this fiscal year:

- 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 are being studied in rodents (Dr. Dieter).

- The toxic effects of organophosphate pesticides and hexane on the central nervous system and blood are being studied at Duke University in collaboration with Dr. Abou-Donia (Dr. Abdo).

- The toxic effects of phthalate esters on energy coupling in liver mitochondria are being studied at NIEHS in collaboration with Dr. Schiller (Dr. Melnick).

- The effects of neurotoxic agents on the in vitro activity of microtubules are being studied at NIEHS in collaboration with Dr. Chignell (Dr. Irwin).

- The in-house research efforts are being directed to assess the sensitivities and versatilities of various tests for detecting subtle kidney injury; to understnad 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 studies from functional and mechanistic viewpoints (Dr. Kluwe).

- The biochemical toxicity of mercuric chloride, promethazine, asbestos and polyvinyl propylene in macrophages and lymphoid organs of rodents were documented to complement contract studies on these chemicals (Dr. Dieter).

Personnel: The branch is organized into two groups - one group is headed by Dr. Dieter with Dr. Irwin, Dr. Melnick and Dr. Abdo and the other group is headed by Dr. Chhabra with Dr. Mennear, Dr. Kluwe and Dr. Dunnick.

Other Activities:

Dr. Kluwe: Prepared a review of available toxicology information on ortho-phthalate esters and organized and participated in a conference on phthalate esters. Developed future research and testing plans on phthalates for NTP.

Dr. Melnick: Was an invited speaker for Phthalate Ester Conference.

Dr. Dunnick: The results of chronic toxicity studies are being compared and correlated to a variety of *in vitro* tests to determine to what extent the *in vitro* tests can be used to predict or substitute for animal tests. Dr. Dunnick and Dr. Melnick organized a seminar series covering topics on various aspects of toxicity evaluation.

Dr. Mennear: Organized a course, "Special Problems in Toxicology" for the N.C. Society of Toxicology Education Committee. Dr. Mennear organized and chaired the first scientific symposium during the first annual meeting of N.C. Society of Toxicology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21000-01 CTEB

PERIOD COVERED
October 1, 1980 to April 30, 1981

TITLE OF PROJECT (80 characters or less)

Evaluation of Clinical Chemistry Used in NTP Standard Protocol

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

PI:	Michael P. Dieter	Physiologist	CTEB/TRTP	NIEHS
Other:	Ralph Wilson	Technician	CPB/TRTP	NIEHS
	Daryl Coston	Technician	CTEB/TRTP	NIEHS

COOPERATING UNITS (if any)

Systemic Toxicology Branch and Chemical Pathology 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 (200 words or less - underline keywords)

Effects of immunotoxic chemicals on non-target organs are being assessed by evaluating a battery of clinical chemistry tests utilizing serum samples from B6C3F1 mice. Some assays that have been found most useful and reliable include serum glutamic pyruvic transaminase, sorbitol dehydrogenase, creatinine, and cholinesterase. We are currently evaluating the utility of serum creatinine kinase, alpha-hydroxybutryic dehydrogenase, acid and alkaline phosphatase and lactate dehydrogenase to detect general or specific organ toxicity.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Current biochemical methods utilizing microvolumes suitable for centrifugal analysis spectrometry are utilized.

MAJOR FINDINGS AND PROPOSED COURSE: More than a half dozen chemicals have been evaluated this fiscal year in the immunotoxicity program. We have performed a variety of clinical chemistry assays to evaluate responsiveness to toxic chemicals, reproducibility, sensitivity, prognostic value for target organ toxicity, and practicality for contract laboratory use. Some of those assays that have been found most useful and reliable include serum glutamic pyruvic transaminase, sorbitol dehydrogenase, creatinine and cholinesterase. Some assays that have been discontinued include serum gamma-glutamyl transferase, ornithine carbamyl transferase, cholesterol and triglycerides. We are currently evaluating the utility of serum creatine kinase to detect muscle and brain toxicity, alpha-hydroxy butyric dehydrogenase for heart toxicity, acid and alkaline phosphatase for bone and intestinal toxicity, and lactate dehydrogenase for general toxicity. The effects of species, sex and age on clinical chemistry parameters are being evaluated at NTP and in contract labs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a sensitive, specific and reliable battery of clinical chemistry assays is a non-destructive technique to evaluate target organ toxicity of a wide range of xenobiotics in rodents and humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21001-01 CTEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanisms of chemical nephrotoxicity

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

PI.: William M. Kluwe Pharmacologist, CTEB/TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/4	3/8	3/8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Time- and dose-dependent effects of selected nephrotoxic agents on the ultrastructure and biochemical status of target and non-target cells in the kidney are evaluated to determine basic mechanisms of injury to various renal cell populations. Comparisons are made between chemical structures and the types of subcellular lesions induced, or the target cells affected, to elucidate common pathophysiological sequences of chemically-induced renal cell injury.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: At several times post-dosing and at multiple dose levels (range of non-toxic to maximally-toxic), evaluations are made of organ morphologies by light microscopy and of subcellular organization by transmission electron microscopy. Evaluations are also made at the same times of biochemical and physiological parameters indicative of the status of cell function in general and subcellular organelle (e.g., plasma membrane, mitochondria) lability more specifically.

MAJOR FINDINGS AND PROPOSED COURSE: Many nephrotoxic organohalide compounds that selectively injure cells of the pars recta (S_3) initially cause vesiculation of the cytoplasm in the apical portion of the cell. Later-appearing morphological effects include microbody proliferation, mitochondrial swelling, increased smooth endoplasmic reticulum and aggregation of chromatin at the periphery of the nucleus.

Assessments are being made of ATP concentration, mitochondrial function, pinocytotic reabsorption, lysosomal lability, endoplasmic reticulum integrity and enzymatic activities and incorporation of precursors into RNA, DNA, protein and lipid to correlate the morphological changes with biochemical effects and to suggest mechanisms of action.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Kidney disease is a major cause of debilitation in the U.S. Though the extent of chemicals as causative agents in toxic nephropathy in humans is unknown, animal studies suggest considerable susceptibility of mammalian kidneys to halogenated hydrocarbons and organic amines. Mechanistic studies are necessary to assess experimental animals as models of human response to nephrotoxicants and for the extrapolation of animal safety studies to the human situation.

PUBLICATIONS

Kluwe, W. M. and Hook, J. B.: Effects of Environmental Chemicals on Kidney Metabolism and Function. Kidney Intl. 18: 648-655, 1980.

Kluwe, W. M. and Hook, J. B.: Metabolic Activation of Nephrotoxic Haloalkanes. Federation Proc. 39: 3129-3133, 1980.

Kluwe, W. M. and Hook, J. B.: Potentiation of acute Chloroform Nephrotoxicity by the Glutathione Depletor Diethyl Maleate and Protection by the Microsomal Enzyme Inhibitor Piperonyl Butoxide. Toxicol. Appl. Pharmacol., 1981 (in press).

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.), 1981 (in press).

ADDITIONAL PROJECTS

1. Chemical Manager for the following chemicals:

Agent	Current Testing Phase
Chlorobenzene	Chronic
Bromobenzene	Prechronic
Benzaldehyde	Prechronic
Nitrofurantoin	Prechronic
4,4'-Diamino-2,2'-stibene-disulfonic acid	Prechronic
Methylphenidate	Pretesting

2. Phthalate Ester Toxicology

An evaluation is currently being made of the adequacy of available toxicology information on ortho-phthalate esters. A NTP-sponsored conference on phthalate esters is being planned and guideline proposals for future NTP endeavors in phthalate ester research are being formulated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21002-01 CTEB

PERIOD COVERED
October 1, 1980 to April 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Immunotoxic Chemicals on Intermediary Metabolism of Mouse Lymphoid Tissues

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

PI:	Michael P. Dieter	Physiologist	CTEB/TRTP	NIEHS
Other:	Ralph Wilson	Technician	CPB/TRTP	NIEHS
	Daryl Coston	Technician	CTEB/TRTP	NIEHS

COOPERATING UNITS (if any)

Chemical Pathology 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 (200 words or less - underline keywords)

The correlation between chemically induced immune defects and biochemical defects in lymphoid tissues (thymus, spleen, bone marrow) and specific-populations of lymphoid cells (pleuripotent stem cells, granulocyte-macrophage precursors, T-cells, B-cells, macrophages) are being investigated. Rate limiting enzymes in monophosphate shunt, glycolysis and tricarboxylic acid, marker enzymes in macrophages, and enzymes of heme metabolism are being assayed. Change in substrate flow through these biosynthetic pathways were caused by immunotoxic chemicals and correlated with specific functional defects in the immune system.

PHS-6040
(Rev. 2-81)

METHODS EMPLOYED: Current biochemical methods utilizing conventional UV and centrifugal analysis spectrometry, and radioenzymatic assays are utilized.

MAJOR FINDINGS AND PROPOSED COURSE: Assay of 6 rate limiting enzymes for glucose metabolism via monophosphate shunt, glycolysis or tricarboxylic acid cycle were standardized in bone marrow, macrophages, thymus and spleen. Change in substrate flow through these biosynthetic pathways were caused by immunotoxic chemicals and correlated with specific functional defects in the immune system. Additional enzymes in macrophages served as markers to differentiate between toxic chemical effects in activated and non-activated cells.

I will continue to participate in a screening program for immunotoxic chemicals by providing correlative evidence of metabolic derangement with immune dysfunction in specific populations of lymphoid cells and macrophages. Whenever feasible and promising, mechanistic studies to elucidate the metabolic basis of specific immunotoxicity will be pursued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information regarding the immunotoxicity of each chemical may be useful in therapeutic intervention and will provide another sensitive measure of the potential chemical hazard to man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21011-01 CTEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Comparative Toxicities and Carcinogenicities of C.I. Direct Blue 6 and Benzidine in Rats.

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

PI:	John H. Mennear	Expert	CTEB/ TRTP	NIEHS
Co-PI:	Bhola N. Gupta	Pathologist	CPB/ TRTP	NIEHS
Other:	Martha Harris	Bio. Lab. Tech.	STB/ TRTP	NIEHS

COOPERATING UNITS (if any)

Biometry

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS; NIH Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	0.75 (technician)

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

C.I. Direct Blue 6, a commercially available textile dye, has been shown to produce hepatocellular carcinoma in rats fed diets containing from 1,500 to 3,000 ppm of test chemical. The dye is derived from benzidine, a known hepatocarcinogen in rats, and when administered orally to rats the dye is metabolized to the carcinogen. Hepatocellular carcinomas induced by the dye appeared after only five weeks of treatment, an onset time which is much shorter than has ever been reported for benzidine-induced carcinomas in rats. This observation raises the question of whether the dye-induced carcinomas were mediated through the dye per se, benzidine produced through the biotransformation of the dye, or some other metabolite. When considered on a molar basis, the benzidine equivalents of the dye were far in excess of any dosage levels of benzidine reported in the literature. The objective of this study is to compare the toxicities and carcinogenicities of benzidine and Direct Blue 6 (in molar equivalent doses with respect to benzidine).

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Benzidine and C.I. Direct Blue 6 will be compared with respect to effects on: 1) food consumption, 2) survival, 3) organ function, 4) urinary excretion of benzidine metabolites, and 5) the development of neoplastic and preneoplastic lesions.

MAJOR FINDINGS AND PROPOSED COURSE: The study is scheduled to begin on 4/29/81. There are no results to report at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study is the intramural portion of the National Toxicology Program's benzidine congener dye initiative. The results will be used to facilitate the interpretation of the pharmacokinetics and genetic toxicology portions of the initiative. The results will also aid in the interpretation of the earlier Direct Blue 6 study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES-30100-02 CTEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Toxic Effects of 1,2-Dibromo-3-chloropropane on the Urogenital System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	William M. Kluwe	Pharmacologist, TRTP	NIEHS
Others:	James C. Lamb, IV	Biologist, TRTP	NIEHS
	Bhola N. Gupta	Pathologist, TRTP	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
TRTP/NIEHS

SECTION
Carcinogenic and Toxicologic Evaluation Branch

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7/8	3/8	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The acute and subchronic toxic effects of the pesticide 1,2-dibromo-3-chloropropane (DBCP) and structurally-related compounds are studied from functional and mechanistic viewpoints. A reported chemo-sterilant in humans, DBCP is no longer manufactured in the U.S., but its presence in ground water and on edible imports and its illegal bulk transport into certain areas of the U.S. require its further toxicological characterization. Effects of DBCP on hepatic, renal and reproductive functions are evaluated at several dose levels, after various treatment regimens and under differing conditions such as age, chemical or physical stress and the like. The distribution and disposition of DBCP is being studied in rats, as well as selected aspects of its metabolism and the effects of metabolic modulation on DBCP toxicities.

Comparative toxicities of DBCP and its metabolites are being evaluated to ascertain the toxic chemical moiety.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Toxic effects are being studied in male and female Fischer 344 rats using a variety of functional, biochemical and pathological techniques. Disposition, distribution and metabolism studies are conducted in, or with tissues from, male Fischer 344 rats by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Acute intoxication with DBCP causes dose-dependent injury to the kidney, testis, epididymis and liver. Effects on the liver, epididymis and kidney appear to be reversible, but testicular damage is progressive and may be irreversible following significant acute injury. The acute toxic manifestations of DBCP treatment bear many similarities with the acute toxic effects of the DBCP metabolites epi- and alpha-chlorohydrin, but not with oxalic acid, another DBCP metabolite. These results suggest that DBCP, epichlorohydrin and alpha-chlorohydrin may exert their effects via a common pathophysiological mechanism. DBCP nephrotoxicity and testicular toxicity is blunted by pretreatment with the microsomal enzyme inducer phenobarbital, but enhanced by pretreatment with cobaltous chloride or by partial hepatectomy. DBCP metabolism, therefore, appears to be involved in the expression of toxicity, though the mechanism of metabolic modulation remains to be elucidated. Repeated exposure to acutely less-than-toxic DBCP doses produces a transient period of infertility in male rats, but no change in epididymal sperm number, motility or morphology. Future studies will continue to characterize the dose-response relationship for DBCP and examine cumulative toxic effects. Pharmacokinetic studies will determine tissue repositories, the relationships of metabolite patterns to tissue injuries and the propensity of DBCP or metabolites to interact with genetic materials.

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) are 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 many 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., 1981 (in press).

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., 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30101-02 CTEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Renal function tests as indicators of nephrotoxicity.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William M. Kluwe Pharmacologist, CTEB/TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 2770

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/8	1/8	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Renal function tests are conducted after single or multiple exposures of rats to a variety of chemical agents to assess the sensitivities and versatilities of the various tests for detecting subtle kidney injury. When appropriate, new or improved methodologies are designed and evaluated. The development of resistance to injury upon repeated chemical exposure and the effect of such or other chemical stresses to the body are also studied.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Following chemical exposure, the animals are placed in metabolic cages and urine is collected under appropriate conditions. Urinalyses of varying completeness are performed to assess the functional status of the kidney. Animals are sacrificed as necessary and the morphological appearance of the kidney compared to the functional effects of chemical treatment.

MAJOR FINDINGS AND PROPOSED COURSE: Tests that measure functional capacities of the kidney are the most sensitive and versatile indicators of subtle kidney injury, while standard tests such as blood urea nitrogen and serum sodium concentrations are relatively poor diagnostic tools.

The resistance to Mercuric chloride ($HgCl_2$) nephrotoxicity induced by repeated treatment with $HgCl_2$ does not extend to other chemical nephrotoxicants that damage the same or dissimilar sections of the proximal tubule as does $HgCl_2$.

Since many nephrotoxicants appear to interfere with protein handling by the proximal tubules, comparisons are being made by electrophoretic methods of the distribution of low molecular weight proteins from the urines of rats treated with selected nephrotoxicants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Sensitive and appropriate endpoints, as are being studied or developed in this project, are essential to the conduct of meaningful toxicology studies, including those conducted by the National Toxicology Program. In addition, sensitive function tests may suggest mechanisms of nephrotoxic action.

PUBLICATIONS

Kluwe, W. M., Renal Function Tests as Indicators of Kidney Injury in Subacute Toxicity Studies. Toxicol. Appl. Pharmacol. 57: 414-424, 1981.

Kluwe, W. M.: Rapid, Automated Measurements of Urinary Protein and Glucose Concentrations. Pharmacol. Meth. 5: 395-400, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30102-02 CTEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Interactions between halogenated aliphatic chemicals and renal tubular cells in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: William M. Kluwe Pharmacologist, CTEB/TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/4	1/8	1/8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemical and physiological functions of renal cells are studied concurrent with, or following, in vitro exposure to nephrotoxic chemicals. The elicited effects are correlated with morphological alterations to assess subcellular mechanisms of action. Parallel studies are conducted in intact animals (in vivo) to assure the relevancy of the effects studied in vitro and to determine the role of extrarenal factors in the development of chemical nephropathy.

The in vitro environment (e.g., pH, electrolytes, cofactors, energy substrates) is manipulated to suggest biochemical mechanisms of action.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparations of renal tissue (e.g., slices, isolated cells or tubules) are combined with nephrotoxic chemicals *in vitro* and several functional (e.g., electrolyte transport, energy metabolism) and biochemical parameters are monitored. The major source of renal tissue is adult, male, F344 rats.

MAJOR FINDINGS AND PROPOSED COURSE: Many nephrotoxic halogenated aliphatic chemicals cause rapid, concentration-dependent depressions of renal proximal tubular cell function *in vitro*. The correlation between functional disturbances produced *in vitro* and *in vivo* is good, though the effects are demonstrable much more rapidly *in vitro* than *in vivo*.

Future experiments will more closely evaluate the morphological effects produced *in vivo* and *in vitro* at early time periods post-exposure and will ascertain the earliest functional abnormalities produced *in vivo*. The metabolism and degradation of toxic organohalides by kidney cells *in vitro* will also be studied and the use of non-rodent (e.g., humans or other primates) tissues will be explored.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Many nephrotoxic halogenated aliphatic chemicals are commonly used as industrial intermediates or as pesticides, resulting in widespread environmental distribution and human exposure. By studying the interactions of these agents with the target cells (kidney proximal tubular epithelium) *in vitro*, mechanisms of action can be suggested, eventually leading to a better estimation of their potential for human injury.

PUBLICATIONS

Kluwe, W. M.: Mechanisms of Acute Nephrotoxicity: Halogenated Aliphatic Hydrocarbons. In Drugs and Environmental Toxicants, (G. A. Porter, ed.), Elsevier-North Holland, 1981 (in press).

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE - Chicago, Illinois 60616
(NIEHS-NO1-ES-5-2156)

TITLE: Long Term Study on the Effect of Ingested Asbestos in Hamsters

CONTRACTOR'S PROJECT DIRECTOR: Alan M. Shefner, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: This contract is for the purpose of studying the long term effects of ingestion (via feed) of asbestos in hamsters. Types of asbestos fibers being studied are short range (fiber size) chrysotile, intermediate range chrysotile and amosite. In addition, low levels of 1,2-dimethylhydrazine (a known intestinal carcinogen) are being used in conjunction with intermediate range chrysotile to study its co-carcinogenic potential.

METHODS EMPLOYED: The above asbestos fibers are mixed in the food at the rate of 1% in the diet and the male and female hamsters are fed this diet for their lifetime. Parameters 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, 1981 all hamsters have died and the histopathology has been completed. The pathology findings have been reviewed by an independent pathologist. The original pathologist's findings and the reviewing pathologist's opinions were reviewed by a NTP Pathology Working Group. The final diagnoses will be completed by May 15, 1981. Results showed that the various forms of asbestos did not affect weight gain or survival. As of May 1, 1981 preliminary pathology results show that the various forms of asbestos did not cause an increase in tumor rates in female hamsters. This information will be available on male hamsters on June 1, 1981. The findings and final report should be completed by July 1, 1981.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas in the United States are contaminated with asbestos fibers as are several common food items. Because of this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 22180
(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: $450,000

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 in rats. The types of asbestos fibers being studied are medium range chrysotile, short range chrysotile, tremolite, crocidolite and amosite. In addition, low levels of 1,2-dimethylhydrazine (DMH) (a known intestinal carcinogen) are being used in conjunction with amosite to study its co-carcinogenic potential.

METHODS EMPLOYED: The above fibers are 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, 1981 all of the rats on the study have died. 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 pathology is being evaluated and a final report completed in FY 82.

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 this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

BATTELLE COLUMBUS LABORATORIES - Columbus, Ohio 43201
NO1-ES-8-2151

TITLE: Comparative Carcinogenicity and Toxicity Studies of Selected Environmental Chemicals in Laboratory Animals Exposed During Pre- and Postnatal Life

CONTRACTOR'S PROJECT DIRECTOR: Arthur C. Peters, D.V.M.

PROJECT OFFICER (NIEHS): Rajendra S. Chhabra, Ph.D., Supervisory Pharmacologist, Toxicology Research and Testing Program (TRTP)

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $949,974

PROJECT DESCRIPTION

OBJECTIVES: The main objective of this study is to determine if there is increased sensitivity of laboratory animals to potential carcinogenic and toxicologic effects of selected environmental chemicals exposed at various dose levels during their in utero development plus postnatal life of two years as compared to the animals exposed only during post-weaning time of two years. This objective will be achieved by (1) studying the carcinogenic potential of selected environmental chemicals exposed to the laboratory animals during their in utero development and weaning period (by treating their mothers with the chemical until weaning) followed by life time exposure (2 years) and comparing it with the effects observed in parallel groups of animals (derived from untreated mothers) exposed to the chemicals only after weaning for two years; (2) studying the effects on reproduction, behavioral, endocrine, and immunological functions at specific time periods during the bioassay in additional groups of animals (incorporated in the bioassay design); and (3) the bioassay will be carried out at 3 dose levels plus controls to determine if there is a dose-response relationship of the chemical for carcinogenic and other toxicologic endpoints.

METHODS EMPLOYED: Under this contract three chemicals are being studied in both male and female B6C3F1 mice and F344 rats. The project is divided into two phases, i.e. prechronic and chronic.

Prechronic Phase of Study: The acute toxicity and MTD (Maximum Tolerated Dose) determination is carried out by following the "Guidelines for Carcinogenic Bioassay in Small Rodents" prepared by the Division of Cancer Cause and Prevention, NCI, NIH.

The Maximum Neonatal Dose (MND) will be determined as follows: mature females (7-9 weeks) will be divided into five groups, i.e. (1) control, (2) MTD (as determined in 90 day studies), (3) 1/3 MTD, (4) 1/10 MTD, and (5) 1/30 MTD. Twelve females per group will be dosed with the chemical (in diet) before breeding. These groups will be bred to untreated, proven fertile males after four weeks or to time of steady state for the chemical and continued on the chemical through weaning of the F_1 generation. The size of litters will be limited to 8 in rats and 6 in mice by random killing of excessive animals on day 4. The MND will be the highest dose at weaning which does not depress the body weight of the offspring more than 10% as compared to the controls; and does not produce mortality, clinical signs of toxicity, pathologic lesions or malformations that would be

predicted to shorten the animals' natural life span. Extra animals will be added in MND determination experiments to analyze the chemical and/or its metabolite(s) concentrations in the tissues and body fluids by using standard analytical GLC or mass spectrometry methods.

Chronic Phase: In addition to the development of tumors as an end point, the appropriate toxicity tests, general histopathology, behavioral and immunology function tests will also be performed on parallel sets of animals placed on same dose regimen at specific time intervals during the test period. The chronic phase will begin with 4 groups of sexually matured (7-9 weeks) females of both species; groups of 90 animals will receive MND of the chemical; two groups of 30 animals in each will receive 1/3 MND and 1/10 MND respectively; fourth group of 90 animals will not receive any treatment. The dosing will begin 4 weeks later, or to the time of steady state for the chemical, before breeding of all groups. Three days before anticipated delivery, the animals will be transferred to a suitable cage to litter. The F_0 females will continue to receive the test chemical while nursing their litters.

A. Carcinogen Bioassay - At weaning of above four groups, not more than 2 males and 2 females, shall be selected randomly from each litter to obtain the total required for the carcinogen bioassay. Eight groups (16 for both sexes) consisting of 50 offspring in each (derived from F_0 mothers) will be treated with test chemical for 2 years as outlined below.

F_0 Treatment Group	F_1 Offspring Randomized Grouping	F_1 Treatment
	———	MTD
MND	———	1/3 MTD
	———	No treatment
1/3 MND	———	1/3 MTD
1/10 MND	———	1/10 MTD
	———	MTD
untreated	———	1/3 MTD
	———	Control

For evaluation of carcinogenic potential the contractor will follow specific toxicopathologic procedures suggested by NIEHS.

B. General Toxicology Tests - A number of tests will be performed on separate animals incorporated in the carcinogen bioassay design. These animals will be exposed to the test chemical at the same dose regimen as that of carcinogen bioassay groups. Various toxicologic endpoints to be tested are described below.

I. Toxico-Pathologic Evaluation - A parallel set of 8 groups of each sex shall be set up. These groups will consist of 10 male and 10 female animals at each test level. Each group shall consist of one F_1 male

and one F_1 female randomly selected from each 10 litters. These groups will be placed on the appropriate treatment at weaning and sacrificed at 9 months of age for toxico-pathologic evaluations which include gross pathology, histopathology, clinical chemistry and tissue levels of the test chemical.

II. Reproductive Function Tests - The animals of the Toxico-Pathologic group, prior to their sacrifice, will be subjected to reproductive function tests.

III. Immune-Function Tests - One additional set of eight groups will consist of 12 males at each test level. Each group will consist of one F_1 male randomly selected from each of 12 litters at weaning and placed on the appropriate treatment. The animals will be sacrificed at 9 months for immune-function tests such as: T-cell function, in vitro by assessing the response of splenic or peripheral blood lymphocytes to mitogen conconavalin A and/or phytohemagglutinin; in vitro B-cell function by assessing its in vitro response of lymphocytes to poke weak mitogen or E. Coli lipopolysaccharide; antibody response to T-dependent antigen by plaque assay; delayed hypersensitivity reaction; and quantitation of immunoglobulin.

IV. Behavioral Tests - A battery of behavioral tests will be applied to 10 male rats per treatment groups from 10 separate litters and 20 male controls from separate litters. The behavioral tests will be performed in the same animals at 4 weeks, 9 months, and 2 years of carcinogen bioassay groups. The tests will include spontaneous motor activity; presence or absence of autonomic signs and for the appearance of normal or deferred motor and pain reflexes; visual placement responses; forelimb grip strength; hind limb extensor reflexes; startle responsiveness and habituation to a time-locked acoustic signal; and one-way avoidance response.

MAJOR FINDINGS AND PROPOSED COURSE: There were originally four chemicals, i.e. Phenytoin, Ethylenethiourea, Firemaster FF-1 and Kepone, that were selected for study under this contract. However, due to the budgetary constraints, Kepone was withdrawn from this study. The following is the status of study on the individual chemicals:

Phenytoin: All pre-chronic studies have been completed. The histopathologic examination of tissues from MND experiments will be finished soon. The results from the subchronic toxicity study have shown some species differences in toxic response and these results were presented at the Society of Toxicology Annual meeting.

Ethylenethiourea: In the subchronic toxicity study the compound-induced histomorphologic lesions were found in the oesophagus, liver, and thyroid of the mice. The thyroid hyperplasia found in the two highest dosages were seen as a preneoplastic response to ETU exposure. In rats, compound-induced histomorphologic lesions were found in the bone marrow, esophagus, liver, pituitary, nonglandular portion of the stomach and thyroid. The thyroid adenomas were seen in the high dosage groups in both sexes.

The MND studies have been extended to 9 weeks for this compound to determine if the carcinogenic potential of this chemical and can be detected at an early age.

Firemaster FF-1: The facilities modification for performance of the PBB study has been completed. The facilities have been inspected by the NTP representatives and were found to be generally satisfactory but needed some minor adjustments.

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.

DEPARTMENT OF ENERGY - BROOKHAVEN NATIONAL LABORARTORY
(NIEHS Interagency ES-9-0043)

TITLE: Evaluation of Repository Mechanics and Other End Points as Indices of Chemical Toxicity

CONTRACTOR'S PROJECT DIRECTOR: R. Drew, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director, National Toxciology Program

DATE INTERAGENCY INITIATED: July 16, 1979

CURRENT ANNUAL LEVEL: $350,000

PROJECT DESCRIPTION

OBJECTIVES: The interagency is for the conduct of a research program for evaluation of respiratory mechanics and other end points as indices of chemical toxicity. Brookhaven National Laboratory will conduct investigations on six chemicals, two animal species, 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 callogen and elastin; and d) evaluate other selective toxicity parameters such as reproductive capacity, hematoporetic change, organ function through use of serum chemistry.

METHODS EMPLOYED: Established techniques for evaluation of repiratory function tests will be adopted. The cytogenetic techniques including sister chromatid exchange will be used for assessment of in vivo mutagenic potential of the chemicals.

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. Multi-breath 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 observation.

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 was 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. Studies are currenlty underway with chlorine

and we anticipate similar studies using silica and cadmium chloride. These studies will continue throughout FY 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the past decade, significant advances have been made in assessing pulmonary functions 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.

TEMPLE UNIVERSITY SCHOOL OF MEDICINE
Philadelphia, Pennsylvania 19140
(NIH-NOI-CP-15752)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. P. Donald Forbes

PROJECT OFFICER (NCI/NTP): June K. Dunnick, Ph.D., Chemist, Division of Cancer Cause and Prevention, Carcinogenesis Testing Program

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $217,803

PROJECT DESCRIPTION

OBJECTIVES: This contract is designed to investigate the toxicity and/or carcinogenicity of the psoralens with and without uv light. This project will test a variety of promising psoralen compounds and compare the relative toxicities. The toxicity of 8-methoxypsoralen, 3-carbethoxypsoralen, and 5-methylisopsoralen will be studied in the HRA/skh mouse. These chemicals will be analyzed in a 3 month prechronic and one year chronic study. This contractor will provide HRA/skh mice to the other NTP contractors involved in the psoralen project.

METHODS EMPLOYED: Animal studies; pathological analysis of tumors; maintenance of animal colony.

MAJOR FINDINGS AND PROPOSED COURSE: The study was started on March 31, 1981 and there are no results at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus uv light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

BIOASSAY SYSTEMS CORPORATION
Woburn, Massachusetts 01801
(NIH-NO1-CP-15753)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Kenneth S. Loveday

PROJECT OFFICER (NCI/NTP): June K. Dunnick, Ph.D., Chemist, Division of Cancer Cause and Prevention, Carcinogenesis Testing Program

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $157,499

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, 3-carbethoxypsoralen, 5-methyl-isopsoralen and 5-methoxypsoralen in the HRA/skh mouse will be studied. The ability of the four psoralen compounds to induce mutations will be determined.

METHODS EMPLOYED: Radioactive profiles of psoralen compounds in the HRA/skh mouse; tissue distribution of psoralens; short term in vitro tests.

MAJOR FINDINGS AND PROPOSED COURSE: The study was started on March 31, 1981 and there are no results at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus uv light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

HRI ASSOCIATES
Emeryville, California 94608
(NIH-NO1-CP-15756)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Stephen Isaacs

PROJECT OFFICER (NCI/NTP): June K. Dunnick, Ph.D., Chemist, Division of Cancer Cause and Prevention, Carcinogenesis Testing Program

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to investigate the toxicity and/or carcinogenicity of the psoralens with and without uv light. This project will test a variety of promising psoralen compounds and compare the relative toxicities. The interactions of DNA with 8-methoxypsoralen, 3-carbethoxypsoralen, 5-methyl-isopsoralen and 5-methoxypsoralen will be tested. In addition, radiolabelled psoralens will be prepared and made available to the NTP for metabolism studies.

METHODS EMPLOYED: Study of DNA interactions using mass-spectral analysis, NMR analysis, and biological studies; radioisotope work.

MAJOR FINDINGS AND PROPOSED COURSE: The study was started on March 31, 1981 and there are no results at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus uv light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

EMORY UNIVERSITY/VA HOSPITAL
Atlanta, Georgia 30322
(NIH-NO1-CP-15767)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Isaac Willis

PROJECT OFFICER (NCI/NTP): June K. Dunnick, Ph.D., Chemist, Division of Cancer Cause and Prevention, Carcinogenesis Testing Program

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $20,000

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to compare the relative toxicity of 8-methoxypsoralen and 5'-aminomethyl-4,4',8-trimethylpsoralen in HRA/skh mice. These studies will be conducted with and without uv light.

METHODS EMPLOYED: animal studies; pathology/histopathology

MAJOR FINDINGS AND PROPOSED COURSE: There are no major findings at this time as the contract has just begun.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralen plus uv light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

UCLA SCHOOL OF MEDICINE
Los Angeles, Califonia 90024
(NIH-NO1-CP-15768)

TITLE: Prechronic studies for the bioassay of 8-methoxypsoralen and related derivatives

CONTRACTOR'S PROJECT DIRECTOR: Dr. Nicholas J. Lowe

PROJECT OFFICER (NCI/NTP): June K. Dunnick, Ph.D., Chemist, Division of Cancer Cause and Prevention, Carcinogenesis Testing Program

DATE CONTRACT INITIATED: March 31, 1981

CURRENT ANNUAL LEVEL: $20,000

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to investigate the toxicity and/or carcinogenicity of psoralen compounds with and without uv light. This contractor will test 8-methoxypsoralen, 3-carbethoxypsoralen, 5-methylisopsoralen, and 5 methoxypsoralen. These compounds will be tested in the lambda prophage induction test. The effects on psoralens on enzyme activities in HRA/skh mouse skin will be measured.

METHODS EMPLOYED: Short term in vitro assays; enzyme assays (ornithine decarboxylase and S-adenosyl L-methionine decarboxylase).

MAJOR FINDINGS AND PROPOSED COURSE: This study has just begun and there are no results at this time.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Psoralen therapy is currently used to treat a variety of diseases including psoriasis. PUVA therapy (psoralens plus uv light) is being investigated at clinics throughout the United States under an IND from the Food and Drug Administration. This treatment has been associated with some side effects, and the object of this project is to help determine these side effects. The results of these studies will be made available to the FDA, physicians using PUVA therapy, and the public at large. This project is part of the overall mission of the National Toxicology Program to determine the toxicity of drug therapy, and to work with other branches of the government in defining and identifying toxic substances.

CONTRACT AWARDS UNDER BASIC ORDERING ARRANGEMENT -- Bioassay Testing

Title: Toxicity and carcinogenicity testing of selected chemicals by the National Toxicology Program under Contracts with EG&G Mason Research Institute, International Research and Development Corporation, Battelle Columbus, Microbiological Associates and Southern Research Institute.

Objective: The objective of this program is to evaluate select chemicals for toxicological endpoints including cancer in experimental animals.

Methods Employed: This assessment requires prechronic studies and chronic carcinogenic bioassays which are performed according to protocols designed by the chemical manager and approved by the Experimental Design Group. Test chemicals are administered dermally, by inhalation, gavage or in feed to F344 rats and B6C3F1 mice. The prechronic phase is a series of linked studies (acute, 14-day repeated dose and 90 day prechronic), in which special studies may be included for better definition of toxic effects. For prechronic studies groups of 5-10 animals of each sex are used to provide information needed to determine the maximum tolerated dose used in the chronic bioassay. In the chronic studies groups of 50 animals of each sex and species are used as controls or receive dose levels equivalent to the MTD or ½ MTD for 104-107 weeks. Upon completion of the chronic exposure period surviving animals are necropsied and gross and histopathologic examinations are performed on 38 tissues from each animal in the study. The contract laboratories where chemicals have been assigned, the chemicals assigned to each laboratory arranged according to contract number, status of the testing, the identification number, annual level of funding for each contract, the Principal Investigator and the NTP Project Officer are as follows:

EG&G MASON RESEARCH INSTITUTE:

Worcester, Massachusetts

Principal Investigator: Dr. Herman S. Lilja
NTP Project Officer: Dr. Carrie E. Whitmire

Compound	Route	NTP#
Pentaerythritol Tetranitrate [a.1, b]	Feed	C55743
2,6-Xylidine [a.1, b]	Gavage	C56188
H.C. Yellow 4 [a.1, b]	Feed	C56019
Chlorpromazine HCl [a.2, b]	Feed	C05210
4-Hydroxyacetanilide [a.2]	Feed	C55801
Tumeric [a.2, c]	Feed	
1-Amino-2,4-Dibromoanthraquinone [a.3, b]	Feed	C55458
2,4-Diaminophenol HCl [a.3]	Gavage	C60026
Probenecid [a.3]	Feed	C56097
Quercetin [a.3]	Feed	C60102
Hexachloroethane [a.4, b]	Gavage	C04604
Nitrobenzene [a.4, b]	Oral/Dermal	C60082
Titanium Ferrocene [a.4, b]	Gavage	C04502

Contracts	Date Initiated	Current Annual Funding Level
a. 1 NO1-CP-95619-01	June 30, 1980	$342,768
2 NO1-CP-95619-02	August 31, 1980	271,353
3 NO1-CP-95619-03	July 31, 1980	312,878
4 NO1-CP-95619-04	August 30, 1980	332,120

b. special studies

c. substituted for curcumin (NCI No. C60015)

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Mattawan, Michigan

Principal Investigator: Dr. Clifford Jessup
NTP Project Officer: Dr. Marcelina B. Powers

Compound	Route	NTP#
Toluene [a.1, b]	(Gavage	10009-V
	(Inhalation	
Isoproterenol, Hcl [a.1]	(Gavage	10130-N
	(Inhalation	
Dimethyloldihydroxyethylene urea [a.1, b]	Inhalation	10205-P
Coumarin [a.2, b]	Gavage	10104-S
6-Methylcoumarin [a.2, b]	Gavage	10136-L
3,4-Dihydrocoumarin [a.2, b]	Gavage	10113-J
Azodicarbonamide [a.3, b]	(Gavage	10086-W
	(Inhalation	
Isobutyl Nitrite [a.3,b]	Inhalation	10869-J
Carvone [a.4, b]	Gavage	10093-S
Resorcinol [a.4, b]	Gavage	10009-V
Diethylphthalate [a.4, b]	Feeding	10112-F
Mercuric chloride [a.5, b]	Gavage	10133-A
Palladium chloride [a.5, b]	Gavage	10148-D
Monochloroacetic acid [a.5, b]	Gavage	10138-V
Chloramphenicol [a.6, b]	Feeding	10096-E
4,4'-Diamino-2,2'-stilbenedisulfonic acid [a.6]	Feeding	10107-S
Cadinene [a.6]	Feeding	10091-J

	Contracts	Date Initiated	Current Annual Funding Level
a.	1 NO1-CP-05700-1	August 31, 1980	$646,850
	2 NO1-CP-05700-2	August 31, 1980	5,399
	3 NO1-CP-05700-3	August 31, 1980	457,251
	4 NO1-CP-05700-4	October 6, 1980	749,500
	5 NO1-CP-05700-5	August 31, 1980	336,121
	6.NO1-CP-05700-6	August 31, 1980	297,185

b. special studies

SOUTHERN RESEARCH INSTITUTE

Birmingham, Alabama

Principal Investigator: Dr. David Prejean
NTP Project Officer: Dr. Michael P. Dieter

Compound	Route	NTP#
Hexachlorocyclopentadiene [a.1]	Gavage	10125-A
Benzaldehyde [a.1]	Gavage	10087-A
Gamma-butyrolactone [a.1]	Gavage	10090-F
Furan [a.1, b]	gavage	10119-M
Furfuryl alcohol [a.1, b]	gavage	10121-J
Furfural [a.1, b]	gavage	10120-F
Ethylene glycol [a.2, b]	feed	C00920
Pigment Red 3 [a.2, b]	feed	C54922
Red 23 [a.2, b]	feed	C60377
Polysorbate 80 [a.2, b]	feed	C60286

	Contracts	Date Initiated	Current Annual Funding Level
a.	1 NO-1-CP-95651-1	June 30, 1980	$487,449
	2 NO-1-CP-95651-2	September 30, 1980	341,508

b. special studies

BATTELLE, COLUMBUS

Columbus, Ohio

Principal Investigator: Dr. Arthur Peters

NTP Project Officer: Dr. Rajendra Chhabra

Compound	Route	NTP#
P-chloraniline [a.1, b]	gavage	C02039B
Dimethoxane [a.1, b]	gavage	C56213
Ocratoxin A [a.1]	gavage	C56586
Ethylenediamine [a.2, b]	gavage	C60402
Methylolacrylamide [a.2, b]	gavage	C60333
1-Vinyl-3-cyclohexane dioxide [a.2, b]	gavage	C60139A

	Contracts	Date Initiated	Current Annual Funding Level
a.	1 NO1-CP-95653-01	August 31, 1980	$209,296
	2 NO1-CP-95653-02	September 30, 1980	346,263

b. special studies

MICROBIOLOGICAL ASSOCIATES

Bethesda, Maryland

Principal Investigator: Dr. Marshall Dinowitz
NTP Project Officer: Dr. Charles K. Grieshaber

Compound	Route	NTP#
dl Amphetamine sulfate [a]	feed	C55710
Sodium Azide [a]	feed	C06462
Tris (2 chloroethyl) phosphate [a]	gavage	C54751

Contracts	Date Initiated	Current Annual Funding Level
a. NO1-CP-95650-01	September 29, 1980	$331,101

Major Findings: All compounds are in the prechronic phase of testing, and no significant data is available.

Significance to Biomedical Research and the National Toxicology Program: The National Toxicology Program (NTP) was established to centralize and strengthen the DHEW's activities in toxicological research and testing and to provide the necessary toxicological information needed by research and regulatory agencies. The NTP contracts should expand considerably the toxicological profiles of the chemicals selected for testing, and the results should be useful in establishing limits for human exposure to these compounds.

Proposed Course: Bioassay experiments will be continued through the chronic tests, sacrifice, necropsy, pathology, data reporting, and preparation of Technical Reports.

CELLULAR AND GENETIC TOXICOLOGY BRANCH

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The Cellular and Genetic Toxicology Branch of the National Toxicology Program provides a comprehensive testing and and research effort focused on short-term test systems which have predictive value for chemicals potentially mutagenic and/or carcinogenic in humans. Individual short-term test systems have the capacity to detect the potential (rather than the ability) of a chemical to induce cancer or mutations in humans. Therefore, a variety of tests capable of measuring mutagenicity in microbial cells, and mutagenicity, cytogenetic damage, DNA damage, and transformation in mammalian cells, are used. The results of these tests provide data used in the design and interpretation of the large-scale, long-term animal carcinogenicity, mutagenicity, and toxicity studies conducted by NTP. In order for short-term test results to be successfully used in a predictive sense, several criteria must be defined. These include a knowledge of both the reproducibility and predictive value of individual test results, and the relationship of the endpoint measured to carcinogenicity, mutagenicity or other toxicological effects in vivo. It is anticipated that the application of a complement of tests which meet these criteria will ultimately result in an effective short-term system for chemical screening. An important part of our program is to produce sufficient short-term test data, particularly across test species and chemical classes, to relate short-term test results to known carcinogenicity in animals. Even with the appropriate use of available test systems, however, it is probable that some potential carcinogens (or cocarcinogens) will not be identified, particularly those which do not induce damage leading to observable gene or chromosome mutations. It is, therefore, important to develop new assays capable of detecting those carcinogens not identified by the assays currently in use.

The major portion of the program concerned with testing and test validation is performed through extramural contracts, while research efforts, test development, test modifications, and data management and analysis activities are performed intramurally (see the Laboratory of Genetics section of this report for additional information on the intramural projects).

PROJECTS RELATED TO SOMATIC EFFECTS

Salmonella/Microsome Test System I

CONTRACTS: NO1-CP-65857; NO1-CP-65855; NO1-CP-75858; and NO1-CP-65856

The primary goal of this project is to evaluate and validate, through collaborative studies in several laboratories, microbial mutagenicity assays for their reproducibility and predictive value in assessing the carcinogenic potential of chemical compounds. Coded chemicals have been systematically tested using a standard protocol which used five strains of Salmonella tymphimurium (TA1535, TA1537, TA1538, TA98, and TA100) in the presence and absence of metabolic activation conditions. The S-9 preparations used for metabolic activation were derived from the livers of non-induced and Aroclor 1254 induced mice, rats, and hamsters. Testing has been completed on approximately 130 coded compounds (some coded compounds being the same chemical under different codes). Of these, approximately 90 have been decoded. The testing results have shown excellent qualitative agreement with results reported in the literature. The few exceptions seem to be

due to chemicals whose mutagenic dose, found in the literature, was above the maximum dose that was called for in the standard protocol. This assay has been found to be quite reproducible in this laboratory, as indicated by responses for chemicals tested at different times under the same code. A good qualitative correlation among the four participating laboratories has also been observed. The proposed course of this contract will be to finish the testing of compounds and enter this data into acomputer data base. Once this has been completed for all the labs involved in this collaborative study, an overall evaluation can be compiled on: in-lab variation, variation between the four labs, comparison of activation systems, and comparison of *in vivo* and microbial test results.

Salmonella/Microsome Test System II

CONTRACTS: NO1-ES-9-2136; NO1-ES-9-2137; and NO1-ES-9-0001

The purpose of these contracts is to test a total of 1125 chemicals for mutagenic potential. Based upon results in *Salmonella*, chemicals are selected for further testing in *Drosophila* (mutagenicity and aneuploidy), mammalian cells (cytogenetics, transformation) and other toxicity tests. *Salmonella* strains TA98, 100, 1535 and 1537 are used in a modification of the Ames assay. All chemicals are incubated with the tester strains in suspension prior to the addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation is provided by liver S-9 preparations from Arochlor 1254-induced Sprague-Dawley rats and Syrian hamsters. All chemicals are tested blind at five doses, in triplicate, in each strain. Also, all chemicals are retested at least 2 weeks later. Results have been received on more than 380 test samples (representing over 280 unique chemicals) and it is anticipated that an additional 170 samples will be completed this calendar year and that 300 samples will be tested per year in future years.

In Vitro Mammalian Cell Mutagenesis

CONTRACTS: NO1-CP-65853 and NO1-CP-65854

The purpose of these contracts is to evaluate and determine the usefulness and reliability of an *in vitro* mutagenesis assay system using L5178Y mouse lymphoma cells (TK^+/-locus) as a prescreen for potential chemical carcinogens. The project is being run as a collaborative study between two laboratories. The project has proceeded in four phases; the first three phases were concerned with the development of the L5178Y TK^+/-mouse lymphoma *in vitro* forward mutation assay protocol, and the fourth phase in validation of the developed assay using coded chemicals. We are now basically concerned with phase four, the blind testing of compounds for the purpose of validation. To date approximately forty compounds are in various stages of testing. These chemicals include ultimate carcinogens, procarcinogens, noncarcinogenic analogs, and chemicals of uncertain carcinogenicity. Evaluations by the two laboratories have shown excellent agreement between labs and results have also shown a high correlation between mutagenic response and the carcinogenic properties of the chemicals. In the future, more compounds will be tested, providing an increased data base for the validation of this system and allowing a more diverse comparison to other *in vitro* assays.

Transformation BALB/c-3T3 Cells

CONTRACT: NO1-CP-55711

The purpose of this contract is to develop and standardize methods for performing in vitro transformation assays with BALB/c-3T3 cells. This assay is used to measure the ability of chemical agents to induce alterations in a population of cells (derived from mouse embryo fibroblasts) from a pattern of controlled monolayer growth to one exhibiting clonal foci of disorientation, piled up growth against a background monolayer. Addition of mouse S-9 showed no beneficial effects in the activation of the system. The testing of 70 coded samples has revealed a good correlation with in vivo carcinogenic activity. Aromatic amines, nitrosamines and synthetic hormones such as diethylstilbestrol showed no transforming activity. The proposed course of this contract will be fourfold: 1) refine and implement means for activation of chemicals requiring metabolism by enzymatic processes either not present or present only at insufficient levels in target cells; 2) continue identification of factors which influence the assay and determine procedures for their standardization; 3) determine sequential processes in the progression from contact-independent growth of the transformed cells to induction of tumor growth in appropriate host animals; and 4) examine the validity of parameters originally set for the transformation assay using the extensive data accumulated from the coded samples tested.

Rat Hepatocyte DNA Repair Assay

CONTRACT: NO1-CP-55705

The principal goal of this effort has been the use of primary rat hepatocytes in culture to detect chemicals with the potential to induce DNA damage. The technique measures the incorporation of radioactive thymidine during nonreplicative DNA synthesis by either of two (autoradiography or scintillation counting) methods.

Cytogenetics

CONTRACTS: NO1-ES-9-0014 and NO1-ES-9-0013

In Vitro Studies - Bacterial mutation systems detect chemically induced point mutations but are not capable of detecting chromosomal damage. Mammalian cells in culture (Chinese hamster ovary, CHO) allow the detection of chromosomal aberrations and, in addition, can be used to detect sister chromatid exchange which probably reflects other types of chromosome damage. Chemicals found mutagenic in this system, regardless of the effect in Salmonella will be given priority for chronic toxicological and carcinogenesis testing. A total of 250 chemicals will be tested in two laboratories (Litton Bionetics, Inc., and Columbia University) over the three year duration of the study.

In Vivo Studies - Assays for induction of chromosomal aberrations and sister chromatid exchange are two in vivo genotoxicity systems in which the methodology has been well developed and exists in many laboratories throughout the world. Although they are laborious and require skill, they offer a significant advantage in that exogenous metabolic activation is not needed. The purpose of this project will be to standardize the testing techniques and obtain data for

comparison with the other short-term test systems to permit evaluation of the relative value of the assays as primary mammalian in vivo genotoxicity tests. It is estimated that two laboratories will participate in a three-year evaluation.

PROJECTS RELATED TO HERITABLE EFFECTS

Drosophila

CONTRACTS: NO1-ES-9-0012; NO1-ES-9-0015; and NO1-ES-9-0016

The ongoing Drosophila mutagenesis effort centers on testing chemicals for heritable effects (sex-linked recessive lethals and reciprocal translocations). Three contract laboratories, each capable of studying 20 coded chemicals per year, are participating in this effort. Validation activities are superimposed on the testing sequence whereby randomly selected coded chemicals are tested in more than one laboratory to insure interlaboratory reproducibility of results.

Aneuploidy

CONTRACT: NO1-ES-1-5002

Assays for the detection of chemically induced aneuploidy, an event not identified by other mutagenesis test systems, will be studied to develop more rapid methods for larger scale testing programs. Aneuploidy assays would, when added to other validated short-term test methods, allow a more comprehensive evaluation of human genetic risk. The insect (Drosophila) and yeast (Sacchromyces) systems allow species comparison, and can be used to test for aneuploidy in both somatic and germinal cells. The two test development/validation contracts will be primarily developmental in the initial year; whereas validation and testing of 50 to 80 chemicals, respectively, will commence in the later years of the 3-year efforts.

SUPPORT ACTIVITIES

Three resource activities exist which can be utilized throughout the mutagenesis program. The chemical repository resource purchases, analyzes, distributes, and stores all chemicals selected for testing. The data system captures, will analyze, stores, and displays testing/validation/development results. The Environmental Mutagen Information Center collects, organizes, stores, and disseminates published information on chemicals tested for mutagenicity.

SRI INTERNATIONAL
Menlo Park, California 94025
(NO1-CP-55701)

TITLE: Development of Detailed Methods and Protocols for Carcinogenicity Screening Using Cell Culture Assays--TASK IV: Hamster System

CONTRACTOR'S PROJECT OFFICERS: Dr. Gustave Freeman
Dr. Douglas W. Fodge

PROJECT OFFICER (NCI/NTP): William J. Caspary, Ph.D., Biochemist, Cellular and Genetic Toxicology Branch, NTP

DATE CONTRACT INITIATED: June 30, 1975

DATE CONTRACT TERMINATES: June 30, 1981

CURRENT ANNUAL LEVEL: $63,407 for FY 1980 and FY 1981

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the potential of a hamster *in vivo* - *in vitro* assay for use in assessing chemical carcinogenicity.

METHODS EMPLOYED: Three areas of related work have been conducted using Syrian hamsters:

1. Pregnant hamsters are exposed to test chemicals by intraperitoneal injection. The transplacentally exposed offspring are observed for tumors throughout their lives.
2. Pregnant hamsters are exposed to test chemicals by intraperitoneal injection and, three days later, primary cell cultures are established from the transplacentally exposed embryos. The frequencies of transformants are then determined in the cultured cells.
3. Primary cell cultures are established from hamster embryos and exposed to test chemicals *in vitro* and the frequency of transformants determined.

Preliminary observations from *in vivo* studies with carcinogenic polyaromatic hydrocarbons indicate an excess of tumors in both treated females and the transplacentally exposed offspring. Final necropsies and histopathologies are presently being performed.

In over 20 studies with 12 chemicals involving cells cultured from embryos taken from carcinogen exposed females, transformed cells were detected only once in a dimethylbenzanthracene experiment.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The *in vivo* - *in vitro* cell culture system has not yet proven to be predictable for screening chemicals for carcinogenicity. These investigations may provide new information about the nature of transplacental oncogenesis, a subject of growing importance and intensive interest.

LITTON BIONETICS, INC.
Kensington, Maryland 20795
(NO1-CP-65853)

SRI INTERNATIONAL
Menlo Park, California 94025
(NO1-CP-65854)

TITLE: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis

CONTRACTORS' PROJECT DIRECTORS: Dr. Brian C. Myhr, Litton
Dr. Ann D. Mitchell, SRI

PROJECT OFFICER (NCI/NTP): William J. Caspary, Ph.D., Biochemist, Cellular and Genetic Toxciology Branch

DATE CONTRACTS INITIATED: September 30, 1976

CURRENT ANNUAL LEVEL: Litton: $288,243
SRI: $305,222

PROJECT DESCRIPTION

OBJECTIVES: To evaluate and determine the usefulness and reliability of an in vitro mutagenesis assay system using L5178Y mouse lymphoma cells ($TK^{+}/-$locus) as a prescreen for potential chemical carcinogens.

METHODS: The mouse lymphoma assay is being run in a collaborative study between these two labs. In the protocol which has been set up for these labs, mouse lymphoma L5178Y $TK^{+}/-$ cell cultures are exposed to a test chemical for four hours. These cells then undergo a two day expression period. Following this, 2×10^{6} cells are seeded in soft agar with selection medium (TFT) and the resistant (mutant) colonies are counted after a ten day incubation period. The cloning efficiency is determined by plating 600 cells from the cell suspension in non-selective medium and taking the ratio of surviving clones to initial cells after a ten day incubation period. Acceptability criteria have been set up as a quality control. These criteria reject experiments which lack a significant number of acceptable dose sets, or show deviant solvent or positive controls. Initially experiments are run with no activation and with activation provided by Aroclor 1254 induced liver homogenates from male Fischer 344 rats (S-9 activation). If the assay appears positive, these conditions are sufficient; however, where the result of an assay appears to be negative, experiments with non-induced S-9 activation will also be run. Experimental results are determined based on a set of statistics which estimates the growth of mutant progenitors. Overall assay results for a compound are determined by looking at sets of experimental results within activation conditions (test results).

MAJOR FINDINGS AND PROPOSED COURSE: This project has proceeded in four phases; the first three phases were concerned with the development of L5178Y $TK^{+}/-$ mouse lymphoma in vitro forward mutation assay, and the fourth phase in validation of the developed assay using coded chemicals. We are now basically concerned with phase four, the blind testing of compounds for the purpose of validation. To date approximately forty compounds are in various stages of testing. These chemicals include ultimate carcinogens, procarcinogens, noncarcinogenic analogs, and chemicals of uncertain carcinogenicity. Evaluations by the two laboratories have shown excellent agreement between labs and results have also shown a high correlation between mutagenic response and the carcinogenic properties of the

chemicals. In the future, more compounds will be blindly tested, providing an increased data base for the validation of this system and allowing a more diverse comparison to other *in vitro* assays.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal carcinogenesis test systems, there is a need for rapid short-term tests, such as the mouse lymphoma mutagenesis assay system, that may be used in a battery for the initial evaluation of chemicals for possible carcinogenic potential.

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
(DOE IAA 40-1-1145-80)

TITLE: Assay of Specific Sequence Transposition in Mammalian Cells

CONTRACTOR'S PRINCIPAL INVESTIGATOR: Dr. Wen Yang

PROJECT OFFICER (NCI/NTP): Raymond W. Tennant, Ph.D., Chief, Cellular and Genetic Toxicology Branch, NTP

DATE CONTRACT INITIATED: October 15, 1980

CURRENT ANNUAL LEVEL: $200,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to develop a specific molecular probe involving the "long terminal repeat" region of the genome of the endogenous retrovirus of RFM strain mice. Since cell to cell transfer of this endogenous virus is genetically restricted in this strain, the probe will be used to establish the specific virus locus by restriction enzyme mapping in normal cells and to identify potential new sites in neoplastic cells which may arise through transposition.

METHODS EMPLOYED: Normal and neoplastic RFM strain cells are cultivated in vitro, with particular emphasis on hematopoietic tissues. Virus induction, isolation, biological cloning and characterization precede the development of a cDNA probe by in vitro reverse transcription. The probe is used in molecular hybridization to restriction-enzyme fragments of cellular DNA to identify specific virus loci. Loci are molecularly cloned using recombinant DNA techniques in E. coli K12 strains.

MAJOR FINDINGS AND PROPOSED COURSE: The endogenous virus of RFM mice (Rfv-1) has been induced, isolated and characterized as to host range properties. Cells established from RFM embryo tissues were isolated, biologically cloned, and used to establish hybrid cell clones with selectively marked hamster cells in order to genetically map the viral locus. Endogenous viral sequences have been partially identified by molecular hybridization using an ecotropic cDNA probe, and reagents for molecular cloning of the viral genome have been developed. Ongoing and future studies will include completion of mapping the virus locus (loci) by genetic and molecular techniques, molecular cloning of a specific sequence probe, and subsequent hybridization to cloned transformed cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: There is considerable evidence that the genetic effects of toxic or carcinogenic agents involves alteration of specific gene elements or genetic mechanisms which are regulatory in nature. Particular emphasis has been placed on transposable genes and mechanisms of gene transposition in mammalian cells. Information concerning these mechanisms and their relationship to neoplastic transformation is a prerequisite for the technical development of an assay system which can utilize these advances.

LITTLE, ARTHUR D., INCORPORATED - Cambridge, Mass. 02140
(NO1-CP-55711)

TITLE: Development of Detailed Methods and Protocols of Carcinogenesis Screening Using Cell Culture Assays - Task II - BALB/c-3T3 Cells.

CONTRACTOR'S PROJECT DIRECTOR: Dr. Andrew Sivak

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $324,732 for FY-80 and FY-81

PROJECT DESCRIPTION

OBJECTIVES: To develop and standardize methods for performing in vitro transformation assays with BALB/c-3T3 cells.

METHODS: The BALB/c-3T3 neoplastic transformation assay was used to measure the ability of chemical agents to induce alterations in a population of cells (derived from mouse embryo fibroblasts) from a pattern of controlled monolayer growth to one exhibiting foci of disorientation, piled up growth against a background monolayer. Toxicity is used to determine the highest dose (10 -20% cell survival) at which a test chemical will be tested. A test chemical that induced a doubling or higher transformation frequency as compared to the controls was considered positive.

MAJOR FINDINGS AND PROPOSED COURSE: A number of factors that affect the quantitative assesments of transformation were examined. The results showed that the survival of cells in mass culture is invariably greater at toxic doses than one would expect from extrapolation of direct cloning data. Also, the number of cells surviving till the end of treatment was dependent on the source of insult and was not reflected in the direct cloning data. These observations suggest that the use of direct cloning data to estimate the number of cells at risk in the transformation assay grossly underestimates the actual population of cells exposed to the test chemical.

A study was conducted to correlate anchorage-independent growth and in vivo tumorogenicity with the progression of Type III foci in cell passage. It was found that the transformed population (Type III foci) acquired the ability to grow in soft agar at the earliest passage. However, although most Type III cell populations grew tumors in irradiatd syngeneic animals, the cell populations did not exhibit quantitative correlation of growth in soft agar and tumorogenicity. Tumorogenicity of transformed populations, contrary to growth in soft agar, was dependent on cell passages in culture. This suggests that populations of clone I-13 contain premalignant cells, which develop tumorogenic potential after extensive passage in culture.

Due to the fact that growth in soft agar was not found to be a consistent marker of oncogenicity for BALB/c-3T3 cells another proposed marker, basal cathepsin B activity, was examined. This marker however was not found to correlate with either the ability of populations to grow in soft agar or to grow tumors in animals.

In comparisons run between cells of the I-13 clone and other cells there were varied results. Cells from the I-11 clone showed no spontaneous background transformation frequency, but were extremely less sensitive to test chemical treatment. Clone I-11 cells also showed little effect when their enhancement with a promoter was attempted. A comparison of the metabolism of MCA by I-13 and C3H-10½ cells showed differences in kinetics between the two. However, no simple relationship between the rate of MCA metabolism and tranformation by MCA could be obtained.

Studies done to provide the BALB/c-3T3 cells with activation showed some success in that aroclor induced adult rat hepatocytes increased the sensitivity of the system to two procarcinogens (MCA and 2-AF). Hepatocytes, however, proved to be very toxic to the target cells at all cell densities, and in some experiments hepatocytes alone increased the transformation frequency. Addition of mouse S-9 showed no beneficial effects in the activation of the system.

The testing of seventy coded samples which have been completed using the BALB/c-3T3 system has revealed a good correlation with in vivo carcinogenic activity of the chemicals tested. Aromatic amines, nitrosamines and synthetic hormones such as diethylstilbestrol showed no transforming activity.

The proposed course of this contract will be fourfold: (1) refine and implement means for activation of chemicals requiring metabolism by enzymatic process either not present or present only at insufficient levels in target cells; (2) continue identification of factors which influence the assay and determine procedures for their standardization; (3) determine sequential processes in the progression from contact independent growth of the transformed cells to induction of tumor growth in appropriate host animals; (4) examine the validity of parameters originally set for the transformation assay using the extensive data accumulated from the coded samples tested. The planned termination date for this contract is September 29, 1981.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The carcinogenic risk to man from chemical agents now present in the environment or being introduced is largely unknown. The task of testing these materials by the classic chronic animal exposure protocols is formidable and prohibitive with respect to resources. While the short-term screening tests for mutagenicity with microorganisms are more reasonable in cost, they are at best only indicative of some genomic alterations and do not adequately reflect the process of carcinogenesis. The cell culture systems under study should provide standardized procedures to examine chemicals for carcinogenicity in short-term systems of modest cost that have genuine biological relevance to the process of carcinogenesis. Further, these cell culture systems should allow a more detailed explanation of the sequential cellular processes occurring in the development of neoplastic disease.

AMERICAN HEALTH FOUNDATION - Vahalla, New York 10595
(NO1-CP-55705)

TITLE: Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays - Task V - Epithelial Cells.

CONTRACTOR'S PROJECT DIRECTOR: Dr. Gary M. Williams

PROJECT OFFICER: Dr. William J. Caspary, Biochemist, Cellular and Genetic Toxicology Branch

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: No Cost Extension

PROJECT DESCRIPTION

OBJECTIVES: To determine the response of epithelial cell cultures to carcinogens, procarcinogens and non-carcinogenic analogues in order to develop methods and procedures for screening of chemical carcinogens.

METHODS: Four approaches have been defined for assaying the effects of carcinogens on cultured rat liver epithelial cells.

(1) The induction of DNA repair by carcinogens in primary cultures of rat hepatocytes - DNA repair, measured by autoradiographic incorporation of ^{3}H-thymidine (TdR) into the nuclei of cultured hepatocytes, has been developed as a screening test for chemical carcinogens. This test which is called the hepatocyte primary culture HPC/DNA repair test, uses density gradient centrifugation to verify that the unscheduled DNA synthesis seen by auto-radiography is DNA repair. This is accomplished by monitoring the incorporation of ^{3}H-Tdr into isolated nonreplicated DNA. Substitution of deoxycytidine for TdR increases the sensitivity of this technique.

(2) The second approach makes use of the induction of hypoxanthine-guanidine phosphoribosyl transferase-deficient mutants in long-term cultures of adult rat liver epithelial cells (ARL). Four ARL cell lines were used and mutagenesis of the treated cells was compared to that of a control.

(3) The adult rat liver cell line 18 (ARL 18) was used to measure sister chromatid exchanges (SCE). Sister chromatids were differentiated by incubating them for 48 hours following the exposure to the carcinogens in a meduim supplemented with 12ug of bromodeoxyuridine (BUdR) and then harvested with vinblastine. Air dried slides were stained with fluorescence plus Giemsa technique to allow SCE detection.

(4) Three cell markers and three population markers which had been previously shown to be reliable indicators of transformation in epithelial cells were used in testing chemicals. The population markers used were gamma-glutamyl transpeptidase (GGT) activity, 2-deoxy-D-glucose uptake, and growth in liquid medium. The three cell markers used were GGT activity detected by cytochemical staining, growth capacity in low Ca^{++} medium, and growth in soft agar.

MAJOR FINDINGS AND PROPOSED COURSE: The potential of the HPC/DNA repair test as a screen for chemical carcinogenesis has been demonstrated; 42 of 44 activation dependent carcinogens from 9 structurally different classes were positive, whereas 16 of 17 non-carcinogenic analogs were negative. Blind testing of compounds in this system is underway and of the 30 compounds tested 12 gave

positive results and 18 gave negative results. It was also found that the HPC/DNA repair test can be modified to detect carcinogens that require activtion by intestinal flora.

A preliminary survey using the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) test using four lines of ARL cells has shown that these lines differ in their metabolic ability to transform chemicals. Thus it may be necessary to use several cell lines in screening so as to provide broad metabolic activation.

The use of the epithelial cell to provide another biological endpoint was investigated. Since ARL 18 appeared to possess the broadest metabolic capability, it was used to measure sister chromatid exchanges (SCE). In testing done on two carcinogens significant dose dependent increases in SCE frequency over a parallel run control were found.

The induction of phenotypic transformation in long-term cultures of rat liver epithelial cells of three population markers and three cell markers have been found to be reliable indicators of transformation in epithelial cells. Study of the growth of cells in soft agar has shown an excellent correlation between this marker and tumorogenicity in newborn rats. For this reason the growth of cells in soft agar has been chosen as the endpoint of the rat liver epithelial transformation assay. In the study of the induction of the markers, gamma-glutamyl transpeptidase cytochemical activity, growth in low Ca^{++} medium, growth in liquid medium, and uptake of 2-deoxy-D-glucose, different carcinogens were found to induce the markers in different sequences.

The proposed course for this contract will be to complete the blind testing of compounds using the HPC/DNA repair test.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The major goal of this project is to develop rapid and reproducible screening assays for chemical carcinogens in epithelial cultures. The HPC/DNA repair test has been validated with a wide variety of chemical classes of carcinogens including samples which were tested blindly. This test can differentiate carcinogens from noncarcinogens. Validations of the ARL/HGPRT mutagenesis assay in several ARL lines reveals the capability to identify carcinogens of 5 chemical classes. Further study of the induction of markers of transformation and the correlation with tumorigenesis should permit validation of this assay. These systems will be of considerable value for evaluating the potential carcinogenicity of chemicals and extablishing priorities for bioassay.

INVERESK RESEARCH INTERNATIONAL
Edinburg, Scotland
(NO1-CP-75858)

LITTON BIONETICS, INC.
Kensington, Maryland
(NO1-CP-65856)

NEW YORK MEDICAL COLLEGE
Vahalla, New York 10595
(NO1-CP-65855)

SRI INTERNATIONAL
Menlo Park, California 94025
(NO1-CP-65857)

TITLE: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

CONTRACTORS' PROJECT DIRECTORS: Dr. Douglas McGregor, Inveresk
Dr. David Brusick, Litton
Dr. Herbert S. Rosenkranz, NYMC
Dr. Kristien Mortelmans, SRI

PROJECT OFFICER (NCI/NTP): William J. Caspary, Ph.D., Biochemist, Cellular and Genetic Toxicology Branch

DATES CONTRACTS INITIATED: Inveresk: December 29, 1976
Litton: September 30, 1976
NYMC: September 30, 1976
SRI: September 30, 1976

DATE CONTRACTS TERMINATE: Inveresk: December 31, 1981
Litton: November 31, 1980
NYMC: May 31, 1981
SRI: November 30, 1981

CURRENT ANNUAL LEVEL: Inveresk: $139,244
Litton: $241,209 (FY 1980 and 1981)
NYMC: $185,918 (FY 1980 and 1981)
SRI: No Cost Extension

PROJECT DESCRIPTION

OBJECTIVES: To evaluate and validate, through collaborative studies in several laboratories microbial mutagenicity, for its predictive value in assessing the carcinogenic potential of chemical compounds.

METHODS EMPLOYED: Coded chemicals were systematically tested using a standard microbial protocol which used five strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, and TA100) in the presence and absence of metabolic activation conditions. The S-9 preparations used for metabolic activation were derived from the livers of non-induced and Aroclor 1254 induced mice, rats, and hamsters. This protocol was also run on the same compounds, under different codes, in all four laboratories.

MAJOR FINDINGS AND PROPOSED COURSE: Tests have been completed on approximately 130 coded compounds (some coded compounds being the same chemical under different codes). Of these approximately 90 have been decoded. The testing results have shown excellent qualitative agreement with results reported in the literature on microbial testing. The few exceptions seem to be due to chemicals whose mutagenic dose, found in the literature, was above the maximum dose that was called for in

the standard protocol. This assay has been found to be quite reproducible, as indicated by responses for chemicals tested at different times under the same code. A good qualitative correlation among the four participating laboratories has also been observed.

The proposed course of these contracts will be to finish the testing of compounds and enter this data in the computer data base (IVIS). Once this has been completed for all the labs involved in this collaborative study, an overall evaluation can be compiled on: within-laboratory variation, variation among the four laboratories, comparison of activation systems, and comparison of *in vivo* and microbial test results. Two laboratories (Litton and SRI) are also developing and validating a modified *Salmonella* protocol that includes a reliable estimate of the toxicity of a chemical. This assay consists of measurements of toxicity and mutagenicity on two separate plates for both a test compound and a toxic non-mutagen in a manner in which the toxicity data from the toxicity plate can be related to the toxicity on the mutation plate.

SIGINFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surrounding. The present study seeks to develop and evaluate rapid, simple and inexpensive, microbial procedures to be used as preliminary screens for chemical carcinogens.

CHEMICAL PATHOLOGY BRANCH

CHEMICAL PATHOLOGY BRANCH
Summary Statement

Mission: During FY 81 the Chemical Pathology Branch continued its three major functions: (1) support of the NIEHS, Comparative Medicine Branch disease surveillance program [5%]; (2) support of the National Toxicology Program, [35%]; and (3) support of Intramural Research [35%] and independent research [25%].

Section Work Areas: Histology, electron microscopy, anatomic pathology and clinical pathology.

Staffing: The Chemical Pathology Branch consists of 6 comparative pathologists, one visiting associate, 11 technicians, one permanent part-time person, 3 secretaries and 2 Stay-In-Schoolers. Dr. Robert Maronpot, formerly of the Carnegie-Mellon University, Pittsburgh, Pennsylvania joined the staff in December 1980 and is serving as head of Experimental Pathology. His duties include directing the Branch's research activities, primarily in regard to developing and validating short term tests for carcinogenicity and studying ways to make current test methods more efficient. Dr. Charles Montgomery, formerly of the Uniformed Services University of the Health Services, Medical School, Bethesda, Maryland, joined the staff in June 1981. He will direct the toxicological pathology group and will be concerned with all aspects of pathology conducted in acute through subchronic studies, especially quality assurance activities. Dr. William Bullock, Division of Laboratory Animal Medicine, Bowman Gray School of Medicine, Wake Forest University, is a consultant for reproductive pathology and laboratory animal pathology. In this capacity he spends about 10 days per year at NIEHS.

Accomplishments:

1. Disease Surveillance Program - The Chemical Pathology Branch conducted 716 necropsies with histopathologic interpretation in support of the disease surveillance program. For details see the report of the Comparative Medicine Branch.

2. Management of Quality Assurance Program for the National Toxicology Program - During FY81 the Chemical Pathology Branch assumed responsibility for evaluating the quality of pathology conducted in bioassays performed by the NTP. This included the review of both 90-day and 2-year studies.

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

   b. review of adrenal lesions of mice, rats and hamsters in an effort to establish uniform diagnostic criteria for use in bioassay studies

   c. review of current histopathologic practices in an effort to reduce the amount of pathology required in bioassay studies

d. monitoring of the pathology aspects of the oral (ingestion) asbestos studies being conducted at the Illinois Institute of Technology Research Institute (hamsters) and Hazleton Laboratories (rats)

e. evaluation of lesions produced by polybrominated biphenyls (180 day studies) in rats and mice plus holding of 180 day animals for lifetime observations

f. evaluation of the comparative toxicity of C.I. Direct Blue 6 and benzidine in rats

g. evaluation of the comparative toxicity of 1,2-dibromo-3-chloropropane and its metabolites, epichlorohydrin and sodium oxalate in male rats

h. evaluation of lesions produced by chlorodecone (kepone) in rats

i. evaluation of lesions produced by inhalation of various forms of asbestos and glass wool in rats; this study is being conducted in conjunction with Dr. Wagner, Pneumoconiosis Research Unit, Medical Research Council, England

j. evaluation of kidney lesions and various clinical chemistry determinations produced by nephrotoxicants in an effort to define appropriate methods of evaluating nephrotoxicants

k. participation in the monthly meetings of the Experimental Design Subgroup of the NCI Bioassay Program

l. evaluation of lesions produced by Agent Orange in rats.

4. Research Program - Independent studies and collaborative efforts with other laboratories in EBB/NTP:

a. evaluation of lesions produced by inhalation of vinyl chloride using various dosing regimens in hamsters, rats and 2 strains of mice

b. immunotoxic and toxic effects of mercuric chloride in mice

c. validate and develop clinical chemistry methods for use in pathology and toxicity methods

d. toxic effects of 1,2-dibromo-3-chloropropane on the urogenital system

e. induction of pulmonary neoplasms following morpholine and nitrogen dioxide exposure

f. evaluation (histological, electron microscopical and histochemical) of preneoplastic lesions in the liver of rats exposed to polybrominated biphenyls and aflatoxin B1

g. assessment of bone marrow toxicity using *in vivo* and *in vitro* culture systems for hemopoietic progenitor cells

h. evaluation of viral pneumonias in rats in an effort to determine the trigger mechanism.

5. Support of the Intramural Research Program - A great deal of support was provided in support of the Comparative Medicine Branch, Laboratory of Reproductive and Developmental Toxicology, Laboratory of Organ Function and Toxicology, Laboratory of Environmental Chemistry, and Laboratory of Pulmonary Function and Toxicology. Lesser support was provided to Laboratory of Biochemical Genetics, Laboratory of Molecular Genetics, Laboratory of Behavioral and Neurological Toxicology and Laboratory of Pharmacology.

a. Comparative Medicine Branch

(1) Dr. A. Edward - a large effort in support of the disease surveillance program. Included routine gross and histopathology of incoming and resident animals in an effort to monitor animal colony health status. Diagnostic pathology of spontaneous deaths

b. Laboratory of Reproductive and Developmental Toxicology

(1) Dr. J. McLachlan - consultations on lesions found in mice exposed *in utero* to diethylstibesterol (DES)

(2) Dr. F.R. Sim - effect of jervine on rat embryos *in vitro*

(3) Dr. J. Hall - morphologic comparison of human spermatozoa from fertile and infertile men

(4) Dr. J. Hall - human sperm penetration of zona-free hamster ova stored at -196°C

(5) Dr. J. McLachlan - electron microscopy and special histologic techniques in the study of the effects of DES on the developing and adult reproductive tract

(6) Dr. S. Harris - R-loop mapping of exona and introns in the natural seminal vesicle; secretion genes IV and V (SVS IV and SVS V)

(7) Dr. S. Harris - heteroduplex mapping of deletions and insertions in the SVS IV and V regions

c. Laboratory of Pharmacology

(1) Drs. C. Shoaf and C. Schiller - lipophilic toxin effects on lipid absorption in the gut

(2) Dr. B. Fowler - several projects involving EM support of studies on the ultrastructural effects of heavy metals on the kidney and liver

d. Laboratory of Pulmonary Function and Toxicology

(1) Dr. R. DiAugustine - neuroendocrine epithelial cells of the guinea pig upper respiratory tract

(2) Dr. Hook - studies on the composition and ultrastructure of abnormal tubular myelin assembly in the lungs of patients with pulmonary alveolar proteinosis

(3) Dr. G. Hook - tubular myelin from the trachea of rabbits

(4) Drs. C. Barrett and E. Sisskin - ultrastructure of cultured epidermal cells as a model for skin carcinogenesis

(5) Drs. C. Barrett and E. Sisskin - cellular and molecular mechanisms of neoplastic progression

(6) Dr. K. Sonstegard - neuroendocrine cells in rabbit fetal lung as a model for in-depth study

e. Laboratory of Behavioral and Neurological Toxicology

(1) Dr. Seth - morphological evaluation of the brain of rats exposed to kepone.

f. Laboratory of Molecular Genetics

(1) Dr. D. Pagano - establish the location of Salmonella typhimurium in the mouse.

4. Publications (Branch Personnel Underlined)

a. Book Chapters and Articles

(1) Biocca, M., Gupta, B.N., Chae, K., McKinney, J.D. and Moore, J.A.: Toxicity of selected symmetrical hexachlorobiphenyl isomers in the mouse. Toxicol. Appl. Pharmacol. (in press) (1981).

(2) Birnbaum, L.D., Decad, G.M., Matthews, H.B. and McConnell E.E.: Fate of 2,3,7,8-tetrachlorodibenzofuran in the monkey. Toxicol. Appl. Pharmacol. 57:189-196 (1981).

(3) Boorman, G.A.: Spontaneous lesions of importance for long-term toxicity testing in rats and mice. In: Second Annual CIIT Conference: Scientific Considerations in Monitoring and Evaluating Toxicological Research (Gralla, E.J. ed.), Hemisphere Publishing Co., New York, pp. 89-121, 1980

(4) Boorman, G.A., Luster, M.I., Dean, J.H. and Luebke, R.W.: Effect of indomethacin on the bone marrow and immune system in the mouse. Clin. Exper. Immunol. (submitted) (1981).

(5) Boorman, G.A., Luster, M.I., Dean, J.H. and Wilson, R.E.: The effects of adult exposure to diethylstilbestrol in the mouse on macrophage function and numbers. J. Reticulo. Soc. 28:547-560 (1980).

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b. Abstracts

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(2) Boorman, G.A.: The effect of indomethacin on the bone marrow and immune system in the mouse. SOT Meeting, San Diego, CA, March 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30089-03 CPB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Efforts to Enhance the Elimination of Polybrominated Biphenyls (Firemaster FF-1) from the Body of Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.E. McConnell	Veterinary Pathologist	CPB/TRTP	NIEHS
OTHER:	J.A. Moore	Supervisory Veterinary Medical Officer	TRTP	NIEHS
	M.W. Harris	Biological Laboratory Technician	STB/TRTP	NIEHS
	R.E. Wilson	Biological Laboratory Technician	CPB/TRTP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Environmental Chemistry, NIEHS

LAB/BRANCH
Chemical Pathology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study was to determine if activated charcoal (AC) or cholestyramine (CSA) had any effect on enhancing the rate of elimination of polybrominated biphenyls (Firemaster FF-1) from the body of rats. Rats were exposed to PBBs in their diet at the rate of 1 mg/kg/day for 6 months, followed by a 4 month recovery period and then 6 months on diets containing either AC or CSA. Parameters studied were body weight effects, histopathology and tissue bromine analysis. Results show that neither AC or CSA reduce the body burden or significantly affect the lesions produced by PBBs.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer strain female rats were given 1 mg/kg polybrominated biphenyls (Firemaster FF-1) in their diet daily for 6 months. They were then continued on a control diet (no exposure) for 4 months. Following this the animals were divided into groups of 6 (exposed) or 9 (controls) and were given charcoal or cholestyramine in their diet with or without periods of restricted caloric intake for 6 months as follows:

Group I	Control	rats	Normal	diet	
II	"	"	"	"	and charcoal
III	"	"	"	"	and cholestyramine
IV	"	"	"	"	and periods of restricted food intake
V	FF-1	"	"	"	
VI	"	"	"	"	and charcoal
VII	"	"	"	"	and charcoal and restricted calories
VIII	"	"	"	"	and cholestyramine
IX	"	"	"	"	and cholestyramine and restricted calories

After 6 months on the above dietary regimen, the animals were killed. Parameters evaluated were body weight gain, organ weights, hematology and clinical chemistry, and chemical analysis of fat and liver for total bromine analysis.

MAJOR FINDINGS AND PROPOSED COURSE: In summary, neither charcoal nor cholestyramine caused a reduction in tissue bromine levels and by inference did not affect PBB levels. In fact, bromine levels did not show a significant reduction in any of the groups suggesting that PBB tissue levels are quite stable. Periods of caloric restriction also failed to reduce tissue bromine levels. This project is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PBBs were responsible for widespread environmental contamination, animal loss and suspected human illness in Michigan during 1973-1974. People in this area still have body burdens of PBBs. It would be extremely useful to find a therapeutic compound which would enhance the elimination of these chemicals from the body.

PUBLICATIONS

McConnell, E.E., Harris, M.W., and Moore, J.A.: Studies on the use of activated charcoal and cholestyramine for reducing the body burden of polybrominated biphenyls. Drug. Chem. Toxicol. 3(3): 277-292, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30105-02 CPB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Assessment of Bone Marrow Toxicity Using In Vivo and In Vitro Culture Systems for Hemopoietic Progenitor Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.A. Boorman	Veterinary Pathologist	CPB/TRTP NIEHS
OTHER:	J.H. Dean	Immunologist	STB/TRTP NIEHS
	M.I. Luster	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Chemical Pathology Branch

SECTION

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 (200 words or less - underline keywords)

While myelotoxicity or bone marrow suppression is a well recognized complication of chemical exposure during cancer chemotherapy, much less attention has been directed towards the effects of environmental agents on the bone marrow. The objective of this study is to examine bone marrow cellularity, pleuripotent hematopoietic stem cells (CFU-S), erythroid precursors, and bone marrow macrophage-granulocyte progenitors (CFU-GM) following exposure to a variety of environmental agents. This study is conducted as part of the immunotoxicology program. Thus, alterations in bone marrow progenitor cells can be correlated with functional alterations in mature cells of the lymphoid system. Studies have been completed to date on over 10 chemicals of environmental concern including polybrominated biphenyl (PBB), tetrachlorodibenzo-p-dioxin (TCDD), diethylstilbestrol (DES), indomethacin, benz(a)pyrene, benz(e)pyrene, and Fryol FR-2. New chemicals being evaluated include those on the NTP Bioassay Program that are potentially immunotoxic as well as others that because of their structure or mechanism of toxicity may aid our understanding of toxicity assessment.

PROJECT DESCRIPTION

METHODS EMPLOYED: Five to six week old female $B_6C_3F_1$ mice are dosed with the chemical to be tested. Following exposure the mice are killed, tissues removed for immunotoxicologic evaluation and the femoral bone marrow cells collected and quantitated. Pleuripotent stem cells (CFU-S) are evaluated by injecting bone marrow cells into irradiated recipients. After 8 days spleen are collected from the recipients and the number of splenic myeloid colonies gives an index of CFU-S content in the bone marrow. IUDR uptake in other recipients is also being run concurrently to assess the size of CFU-S colonies. Donor-macrophage-granulocyte progenitors (CFU-GM) are quantitated by plating bone marrow cells in vitro cultures to which appropriate stimulus has been added. Quantitation of colonies forming after seven days of culture gives an index of macrophage-granulocyte progenitors in the bone marrow cells. The proliferation of bone marrow cells is also measured in liquid culture using microtiter plates and a ^{3}H-thymidine pulse. This additional parameter gives the proliferative potential of the progenitor cells. Erythropoiesis is measured by ^{59}Fe injection and 24 hours later quantitating splenic and femoral radioactivity. Some mice are bled 24 hours prior to ^{59}Fe injection to assess the effect of "anemia stress" on the bone marrow response following chemical exposure.

MAJOR FINDINGS AND PROPOSED COURSE: All chemicals tested to date have resulted in perturbations of bone marrow progenitor cells. Diethylstilbestrol and dioxin caused a marked decrease in both pleuripotent stem cells and macrophage-granulocyte progenitors. Polybrominated biphenyl (PBB) resulted in an increase of CFU-GM's. This may be analogous to chronic low level stimulation of the marrow as has been reported for low levels of cyclophosphamide. With the assessment of a wide variety of chemicals it is now possible to start to look for correlations between bone marrow alterations and other immune parameters and host resistance assays. Current efforts are to determine which chemicals may cause permanent stem cell defects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aplastic anemias and other alterations in bone marrow are not uncommon and in some cases can be related to physical or chemical injury to the bone marrow. This has been especially true in the field of cancer chemotherapy where bone marrow toxicity and late bone marrow failures is an all too frequent occurrance.

SYSTEMIC TOXICOLOGY BRANCH

SYSTEMIC TOXICOLOGY BRANCH

SUMMARY STATEMENT

Complete toxicologic characterization of chemicals in laboratory animals remains as the foundation for predicting with mounting assurance the eventual adverse effects from particular chemicals to humans. These toxicology information dossiers come from the integrated programs within the Systemic Toxicology Branch, and in combination with the experimental data generated in other Branches (Cellular and Genetic Toxicology, Chemical Pathology, Carcinogenesis and Toxicology Evaluation) allow an evaluation to be made on the gamut of toxic effects in animals and form the basis for extrapolating potential hazards to humans.

The Systemic Toxicology Branch comprises five sections: Biochemical Toxicology, Chemical Disposition, Fertility and Reproduction, Immunologic Toxicology, and Inhalation Toxicology. Each is summarized below; for more details and specific accomplishments consult the individual discussions on the following pages.

Biochemical Toxicology--Studies structure-activity relationships of chemicals to ascertain the mechanism(s) of action(s) at the molecular and biochemical level. Major projects encompass structure-activity effects of polychlorinated biphenyls (PCBs) and chlorinated benzenes, purification of cytochromes induced by hexachlorinated biphenyls (2,3,5,2',3',5',-PCB and 3,4,5,3',4',5'-PCB), and effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on arachidonic acid metabolism.

Chemical Disposition--Investigates the absorption, distribution, metabolism, and excretion of a range of chemicals to provide information which will be useful to the design and interpretation of studies of chemical toxicity and carcinogenicity. Studies of chemical disposition are chosen and designed to provide data which will permit a better understanding of the structure-activity relations which determine the rates of chemical absorption, distribution, metabolism, and excretion. Thus these are designed to contribute to our basic understanding of chemical toxicity and will identify those factors which mediate chemical toxicity and will allow a more accurate extrapolation of laboratory data to humans.

Major projects include pharmacokinetics of chlorinated xenobiotics; metabolism and disposition of aromatic amines, halogenated alkyl phosphates, acrylamide, and disazobiphenyl dyes; disposition of halogenated dibenzofurans, chlorpheniramine maleate, and parachloroaniline; and extrapolation of in vitro to in vivo metabolic rates for polychlorinated biphenyls.

Fertility and Reproduction--Provides three major types of support: Maintenance of an in-house research program, consultation to Chemical Managers on the design of special studies, and participation with NIOSH and NCTR personnel in the organization, coordination, and long range planning of the NTP Reproductive and Developmental Toxicology Program.

The in-house research activities include the evaluation of the reproductive toxicity of environmental and industrial chemicals, and provide relevant data on the toxic potential and mechanism of chemicals, enhance the development of new and more appropriate testing systems, and assure technology in test development and validation.

Immunologic Toxicology--Selects, refines, and validates a panel of immunology and host resistance procedures to define immunotoxicity and to correlate changes in immune function with alterations in host resistance. The comprehensive assay panel evaluates: (1) host resistance to bacterial and tumor cell challenge; (2) cell-mediated immune function as measured by lymphoproliferative responses to mitogens; (3) development of delayed hypersensitivity to a T-cell dependent anitgen; (4) assessment of antibody plaque forming cell responses; (5) quantitation of immunoglobulin levels; and (6) bone marrow progenitor cell function.

Chemicals examined for immunotoxicity using the immunology host resistance assays include: benzo (a) pyrene and benzo (e) pyrene, phorbol myristate acetate, methyl carbamate and ethyl carbamate (urethane), and promethazine.

During the past year immunological profiles and clinical evaluations were done on PPB-exposed Michigan dairy farmers and on Michigan chemical workers, along with a low level exposure group from the general population and two negative control groups, one from the Michigan general population and the other from Wisconsin dairy farmers. These data will be analysed and the findings made known during the coming year.

Inhalation Toxicology--Conducts studies on compounds to which toxicologically significant exposure occurs primarily by inhalation and works toward the technologic advancement of gas-vapor inhalation methodologies/facilities. The research program divids into three areas: (1) cardiac toxicology using isolated, perfused hearts and other cardiac tissue, (2) carcinogenic potential of simultaneous exposure to nitrogen dioxide and nitrosatable amines, and (3) inhalation toxicology of environmental chemicals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21003-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Disposition of Halogenated Dibenzofurans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Linda S. Birnbaum	Research Microbiologist	STB/TRTP	NIEHS
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	Gary M. Decad	Staff Fellow	STB/TRTP	NIEHS
	E. E. McConnell	Chief, Chemical Pathology	CPB/TRTP	NIEHS
	Kun Chae	Research Chemist	LEC	NIEHS

COOPERATING UNITS (if any)

Laboratory of Environmental Chemistry

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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 (200 words or less - underline keywords)

Halogenated dibenzofurans are found worldwide as environmental pollutants. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the degree and position of halogenation. This work has established that 2,3,7,8-tetrachlorodibenzofuran (TCDF), an extremely toxic isomer, is excreted only after metabolism and toxicity is inversely related to metabolic capability. Thus, rats and mice, which readily metabolize TCDF, are relatively insensitive to its toxic action. Monkeys, which are more sensitive than rats and mice, metabolize TCDF at a slower rate. Guinea pigs, which are exquisitely sensitive to the toxic action of TCDF, fail to metabolize this compound at all. In fact, the lack of clearance results in accumulation upon low-dose chronic exposure. The concept of a threshold body burden for toxicity is currently being tested. The absorption, distribution, metabolism and excretion of two different hexachlorodibenzofurans (HCDFs) is being investigated in rats. The nature of the metabolites produced from these HCDFs and TCDFs is being examined.

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has used radioactively labeled compounds to quantitate absorption, distribution, metabolism, and excretion of several polyhalogenated dibenzofurans (PHDFs). TCDF was labeled with ^{14}C; the two HCDF isomers, 1,2,4, 6,8,9-HCDF (H19) and 2,3,4, 6,7,8-HCDF (H27) are labeled with ^{3}H. The disposition of TCDF has been studied in rats, two strains of mice, guinea pigs and Rhesus monkeys after acute exposure, and after repeated exposure in guinea pigs. H19 and H27 are being studied in rats.

1,2,4,6,8,9-hexachlorodibenzofuran (H19)

2,3,4,6,7,8-hexachlorodibenzofuran (H27)

2,3,7,8-tetrachlorodibenzofuran (TCDF)

Analysis were facilitated by the use of a biological material's oxidizer and liquid scintillation counting 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 : 1) TCDF is readily absorbed from the gut of both rats and guinea pigs. Independent of the route of administration, it is initially concentrated in the liver of rats, mice, guinea pigs, and monkeys. It is then distributed for storage to the adipose tissue, from which it returns to the liver for metabolism, followed by excretion via the bile into the feces. In the rat, intestinal metabolism of the biliary metabolites may occur in contrast to the situation in monkeys. At least one metabolite from monkey feces has been partially isolated. The profile of biliary metabolites from the rats has been analyzed as to hydrolytic sensitivities and studies are underway to isolate and characterize the major biliary metabolite.

No 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. Further studies to clearly define the threshold dose for toxicity as well as better estimates of the half-life in guinea pigs are underway.

The disposition of two ^{3}H-HCDF isomers is being examined in order to extend the structure/activity relationships for polyhalogenated aromatics previously elucidated by members of this reasearch group, specifically, Dr. H. B. Matthews. The gross toxicity of these compounds is also being investigated. Because of the extent of chlorination and the positions of the halogen atoms, we would expect that H19 should be persistent, while H27 might be more readily metabolized but also more toxic. Preliminary results in rats have shown no toxic effects to an acute exposure of H19. However, this compound is poorly absorbed from the gut. Initially, some metabolism occurs, the products of which are rapidly excreted via the bile. However, this may be due to a small amount of pentachlorodibenzofuran isomer(s), possibly produced by dechlorination during the tritiation process, present in the H19 preparation.

Studies with H27 will be undertaken shortly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Halogenated dibenzofurans are among the most toxic and persistent contaminants in the environment. Some of these chemicals have been implicated in human disorders. We need to be able to predict what the fate of these compounds will be in man. By the better understanding of their disposition in animals, we will be more able to estimate the risk to mankind.

PUBLICATIONS

Birnbaum, L. S., Decad, G. M., and Matthews, H. B.: Disposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat. Toxicol. Appl. Pharmacol. 55: 342-352, 1980.

Birnbaum, L. S., Decad, G. M., Matthews, H. B., and McConnell, E. E.: Fate of 2,3,7,8-tetrachlorodibenzofuran in the monkey. Toxicol. Appl. Pharmacol. 57: 189-196, 1981.

Decad, G. M., Birnbaum, L. S., and Matthews, H. B.: 2,3,7,8-Tetrachlorodibenzofuran tissue distribution and excretion in guinea pigs. Toxicol. Appl. Pharmacol. 57: 231-240, 1981.

Decad, G. M., Birnbaum, L.S., and Matthews, H. B.: Distribution and excretion of 2,3,7,8-tetrachlorodibenzofuran in C57BL/6J and DBA/2J mice. Toxicol. Appl. Pharmacol., In press.

Decad, G. M., Birnbaum, L. S., and Matthews, H. B.: Disposition of 2,3,7,8-tetrachlorodibenzofuran in guinea pigs, rats, and monkeys. In Hutzinger, O., Merian, E., and Frei, R. W. (Eds.): Impact of Chlorinated Dioxins and Related Compounds on the Environment. Oxford, Pergamon Press Ltd., In press.

Decad, G. M., and Birnbaum, L. S.: Noninvasive technique for intravenous injection of guinea pigs. Lab. Animal Sci. 31: 85-86, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21004-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Senescent Changes in Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Linda S. Birnbaum	Research Microbiologist STB/TRTP NIEHS
Other:	Michael Dieter	Research Physiologist CTEB/TRTP NIEHS
	Joyce Goldstein	Research Pharmacologist STB/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:
1.0	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Age-related changes in many physicological parameters have long been known to occur. The basis for these alterations is, however, not well understood. Response to various stresses seems to decline with age. Changes in the ability to metabolize exogenous as well as endogenous compounds has been suggested as a cause for altered functions. This work will explore senescent changes in metabolism of several tissues - liver, lung, kidney, brain, lymphoid tissues. Enzyme systems such as glucuronyl transferase, β-glucuronidase, prostaglandin synthetase, those involved in glucose and heme metabolism, and those involved in intermediary metabolism and immune responses will be investigated. Altered disposition of chemicals in aging animals is being studied in order to elucidate the basis for age-related changes in pharmacological responses.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

This analysis of age-related changes in metabolism can basically be divided into two major divisions, altered pharmacological properties related to the body's ability to handle various drugs and environmental chemicals and alterations in intermediary metabolism in the brain and lymphoid tissues.

METHODS EMPLOYED: A colony of aging male Fisher F344 rats has been established by NIEHS at Charles Rivers Laboratories. Weanling male rats,approximately 15 each month, are placed in the colony to be held until needed. An interium colony of retired breeder F344 male rats has been maintained at NIEHS. As of May 1981, the oldest rats in this group will be 24 months old. This is approximately the mean life span of this strain of rat fed ad libidum and maintained under conventionally clean conditions. Once rats from the Charles River colony approach 2 years of age, the in-house colony will be phased out.

For studies of age-related changes, old animals (>24 months) will be compared to young adult animals (2½-6 mos) and to middle-aged ones (12-16 mos). If necessary, additional ages will be used.

MAJOR FINDINGS AND PROPOSED COURSE:

a) Altered pharmacological responses - Previous investigations by the principal investigator have involved an analysis of age-related changes in hepatic drug metabolism in vitro, specifically alterations in the mixed-function oxidases system. Currently, work is in progress to explore the effects of aging on glucuronyl transferase and β-glucuronidase activity in liver, lung, and kidney. Alterations in the prostaglandin synthetase enzyme system as a function of age are planned in the same tissues.

Changes in the disposition of two polychlorinated biphenyls (PCBs) in aging rats have also been initiated. In young rodents, 2,4,5,2',4',5-hexachlorobiphenyl (245-HCB) is not metabolized and is extremely persistent. On the other hand, 2,3,6,2',3',6'-hexachlorobiphenyl (236-HCB) is readily metabolized and rapidly excreted. We are exploring whether or not senescence alters these pharmacokinetic parameters. One preliminary result suggests that 236-HCB is metabolized more slowly by the aging rat and the metabolites produced are retained in the body to a greater extent.

b) Alterations in intermediary metabolism in the brain and lymphoid tissue - The brain will be used to assay enzymes involved in glucose metabolism. Specifically the cerebral hemispheres and cerebellum will be studied for age-related changes in glucose production and utilization. Degeneration of neuronal function may be caused by age-related modifications in glucose metabolism. The same tissues will also be used to investigate senescent changes in heme biosynthesis and catabolism. It is known that these pathways are influenced by metals whose presence increases with advancing age in the central nervous system.

Lymphoid tissues and associated cells, including thymus, spleen, bone marrow and macrophages, will be examined for age-related changes in intermediary metabolism

that may be associated with functional loss of the immune responses. Enzymes involved in glucose metabolism will be studied as will those involved in functions of antigen recognition and processing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An increasing percentage of our population are senior citizens. Little is known about the causes of biological aging, and possibly less about what really happens. This research project should lay some additional ground work in exploring the biological basis of age-related changes. Enhanced risks or better therapeutic methods may be elucidated by such studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21005-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolism and Disposition of Aromatic Amines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist STB/TRTP NIEHS
	Yiannakis M. Ioannou	Staff Fellow STB/TRTP NIEHS
	Harish Chopade	Visiting Fellow STB/TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	1.8	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the absorption, distribution, metabolism and excretion of two ^{14}C-labeled aromatic amines, p-nitroaniline and diphenyl guanidine, in the rat indicate that clearance rates vary with compound, but that both compounds are readily absorbed, rapidly metabolized and excreted in the form of several metabolites. Very little of either parent compound is excreted prior to metabolism. These compounds do not appear to be accumulated to a significant extent in any tissue, possibly due to the fact that they are rapidly metabolized. Within a few hours after administration most of the dose retained by the body was in the form of metabolites. These compounds were eliminated in both urine and feces, p-nitroaniline primarily in urine, and whole body half-lives for both compounds were less than 1 day. Additional studies of these and other aromatic amines will provide a better understanding of the importance of chemical structure to metabolism, disposition and persistence of aromatic amines.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The absorption, distribution, metabolism, and excretion of a series of aromatic amines is being studied following oral and iv administration to adult male rats. Absorption and distribution to the major tissues and clearance from the tissues into excreta are quantitated by utilizing ^{14}C-labeled compounds, serial sacrifice, sampling and oxidation of samples to $^{14}CO_2$ in a biological material oxidizer for analysis by liquid scintillation counting. Metabolite identification involves solvent extraction of tissues and excreta, purification by thin-layer and high pressure liquid chromatography and co-chromatography with authenic standards. Kinetic parameters are based on the disposition data and are calculated by computer.

MAJOR FINDINGS AND PROPOSED COURSE:

1) p-Nitroaniline was readily absorbed from the gastrointestinal tract distributed to all the tissues by blood and rapidly excreted. p-Nitroaniline was excreted in urine in the form of a number of metabolites. The half-life for clearance from most tissues was less than 12 hours and little p-nitroaniline derived radioactivity remained in the body after 24 hours. Trace levels of p-nitroaniline were most persistent in blood, possibly due to a reaction with hemoglobin. Metabolites of p-nitroaniline are being identified by cochromatography with authenic standards.

2) 1,3-Diphenyl guanidine was readily absorbed from the gastrointestinal tract and distributed throughout the major tissues. There is considerable potential for tissue exposure, but this compound does not appear to bioaccumulate in any particular tissue. Whole body half life for 1,3-diphenyl guanidine is approximately 12 to 16 hours in the rat . Excretion was approximately equally divided between urine and feces.

PROPOSED COURSE: The metabolism and disposition of at least three more aromatic amines, 4-chloro-2-nitroaniline, 2,4-dinitroaniline and 2-bromo-4,6-dinitroaniline, will be studied in the rat. In these studies there will be a special emphasis on the isolation and identification of metabolites which are known carcinogens. Additional goals of these studies will be to observe the effect of halogenation, nitro groups and polarity on metabolism, disposition and bioaccumulation. Further work on aromatic amines will be planned according to the results of these studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE; Aromatic amines are widely used as industrial chemicals, particularly in the rubber and dye industries. Annual use of individual chemicals frequently exceeds one million pounds per year and as a group these chemicals account for many millions of pounds per year. A number of aromatic amines, such as β-naphthalamine, benzidine and 4-aminobiphenyl are known human carcinogens and measures have been taken to remove these chemicals from the market and to minimize industrial exposure. However, the toxicology of the aromatic amines which are currently in use is largely unknown. It is known that on acute exposure some aromatic amines are quite toxic whereas others are relatively innocuous; however, little is known about the metabolism, disposition or persistence of these chemicals and knowledge

of their potential for chronic toxicity is almost completely lacking. Some aromatic amines have structures which suggest that they might be metabolized to known carcinogens or closely related compounds. The present work is designed to isolate and identify metabolites of these chemicals and determine how various groups on the aromatic rings affect absorption, metabolism and disposition of aromatic amines. These studies may identify potentially carcinogenic aromatic amines which are currently in industrial use and should provide a better understanding of those chemical structural factors which facilitate or inhibit absorption, metabolism and elimination of these compounds.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21006-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Chronic Exposure to Airborne Environmental Agents - Vinyl Chloride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	TRTP NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	CPB/TRTP NIEHS
	G.A. Boorman	Veterinary Pathologist	CPB/TRTP NIEHS
	J.K. Haseman	Statistician	BB NIEHS

COOPERATING UNITS (if any)

Becton Dickinson & Co., Research Triangle Park, North Carolina
Experimental Pathology Laboratory, Raleigh, North Carolina

LAB/BRANCH
Systemic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vinyl chloride (VC) was administered via inhalation to groups of female hamsters, rats, and 2 strains of mice using various dosing regimens in an effort to compare the carcinogenic response. The different groups were exposed to VC for 6 hours/day, 5 days/week as follows: 0-6 months, 12-18 months, 12-24 months and 18-24 months following the start (animals were 8 weeks of age at start) of the experiment.

VC related tumors were dependent on both the age of the animal during exposure and duration of exposure. In general, the younger the animal when exposed the higher the incidence of tumors when compared to groups exposed for the same length of time but starting with older animals. Exposures of longer than 12 months did not significantly increase tumor incidence.

PROJECT DESCRIPTION

METHODS EMPLOYED: The vinyl chloride study was designed to compare the carcinogenic response in female hamsters, rats and two strains of mice to various dosing regimens. The hamsters were exposed to 200 ppm VC, mice to 50 ppm VC and the rats to 100 ppm VC; levels known to be carcinogenic for each species. The different groups were exposed as follows: 0-6 months, 0-12 months, 0-18 months, life-time (24 months), 6-12 months, 6-18 months, 12-18 months, 12-24 months, and 18-24 months following the start of the experiment. The animals were 2 months of age at the beginning of the experiment.

MAJOR FINDINGS AND PROPOSED COURSE: In the vinyl chloride studies the rat and hamster tissues have been examined and the results tabulated. In rats there is a dose response increase in angiosarcomas with increasing exposure durations. Rats exposed to 0, 0-6, 0-12, 0-18, and 0-24 months having 2, 5, 21, 26 and 42% angiosarcomas respectively. Exposures at 12-18 and 12-24 months did not result in a significant increase in angiosarcomas, suggesting age at time of exposure is a critical factor. Hepatocellular carcinomas and mammary gland carcinomas were also increased following vinyl chloride exposure. In hamsters, the highest incidence (15%) of angiosarcomas was found in hamsters exposed 0-6 months to vinyl chloride. Increasing exposure duration or beginning later in life results in the production of a lower incidence of angiosarcomas. Mammary gland carcinomas were also increased in hamsters following vinyl chloride exposure, however, 0-6 months exposure appears as effective in producing tumors as does longer exposure regimens. VC was also carcinogenic in both strains of mice, causing similar types of tumors. In both strains the highest incidence of malignant tumors occurred in animals exposed in the first 6 months of the study. More complete statistical evaluation is currently underway. The results will be completed in FY 1981. 100% of the animals in the fiber study have been necropsied.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21007-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Chronic Exposure to Airborne Environmental Agents - Particulates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	TRTP NIEHS
OTHER	E.E. McConnell	Veterinary Pathologist	CPB/TRTP NIEHS
	G.A. Boorman	Veterinary Pathologist	CPB/TRTP NIEHS
	J.K. Haseman	Statistician	BB NIEHS

COOPERATING UNITS (if any)

MRC Pneumoconoisis Unit, Llandough Hospital, Penarth, Glamorgan, Great Britian
Becton Dickinson & Co., Research Triangle Park, North Carolina

LAB/BRANCH
Systemic Toxicology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 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 (200 words or less - underline keywords)

Various forms of chrysotile asbestos (short range, intermediate range, and Canadian Standard B) and glass wool (JM-100) were given to male and female rats in an inhalation chamber 7 hours/day, 5 days/week for 12 months. The rats were then held for life time observation. Groups of animals were killed at 3 months and 12 months exposure and 12 months postexposure to evaluate lung lesions. All forms of asbestos (especially the longer fibers) caused fibrosis in the area of the terminal bronchioles which progressed during exposure and remained static during the postexposure period. The glass wool did not produce this lesion. Pulmonary carcinomas were observed in rats exposed to asbestos.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The fiber (asbestos and glass wool) studies were conducted using male and female Fischer 344 rats which were exposed for 7 hr/day, 5 days/week for 12 months. The animals will then be held for their life-time. The types of asbestos used were short range chrysotile, chrysotile B, and NIEHS intermediate; the glass wool was Johns-Manville 100. The object of the study was to determine and compare the fiber retention in the lung and fibrogenic and carcinogenic potential of these fibers using standard physiological and pathology techniques. An additional morphometric study using electron microscopy is being conducted to study and quantitate the early changes in the lung produced by these fibers.

MAJOR FINDINGS AND PROPOSED COURSE: All of the rats on the life time portion of the study have died. Interim sacrifices at 3, 12, and 24 months were also conducted. Pathology results from the interim animals showed asbestos (pulmonary fibrosis) which was progressive during the exposure phase and remained relatively static during the 12 month postexposure phase. Asbestosis was more severe with the intermediate range chrysotile and chrysotile B than the short range chrysotile. The glass fibers caused only minimal changes in the lung. The pathology on the life time rats is approximately half completed. Preliminary results indicate that both intermediate range chrostile and chrysotile B cause carcinomas in the lung. The short range chrysotile is suspect in this regard, while the JM-100 is negative to date. It expected that this study will be completed in FY 1981.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The information gained from the asbestos studies will have immediate societal impact since the problems associated with the inhalation of asbestos in humans are well known. One of the major forms of insulation presently being used is glass wool, therefore the information gained from this aspect of the study is extremely important.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21008-01 STB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Carcinogenic Potential of Polybrominated Biphenyl Mixture in the Rat and Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	TRTP NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	CPB/TRTP NIEHS
	B.N. Gupta	Veterinary Pathologist	CPB/TRTP NIEHS
	M.W. Harris	Biological Laboratory Technician	STB/TRTP NIEHS
	J.D. Allen	Biological Laboratory Technician	STB/TRTP NIEHS
	D.L. Myers	Biological Laboratory Technician	CPB/TRTP NIEHS
	R.E. Wilson	Biological Laboratory Technician	CPB/TRTP NIEHS
	J.A. Haseman	Statistician	BB NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH

Systemic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polybrominated biphenyl mixture (Firemaster FF-1) was given orally to rats and mice at 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg body weight, 125 total doses over a 6-month period. After the 6-month exposure to PBB, the animals were held for an additional 2-year period for lifetime observation.

Polybrominated biphenyl mixture (Firemaster FF-1) was carcinogenic for Fischer 344 rats and B6C3F1 mice of both sexes. The higher incidence of hepatic neoplasms included neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas in rats and hepatocellular carcinomas in mice. Other toxicities included porphyrogenic effects, hepatotoxicity, chronic progressive nephropathy and hyperplastic gastropathy in the rat. The PBB mixture also affected the body weight gain in male and female rats and male mice although there was no significant difference in food consumption.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Polybrominated biphenyl mixture, Lot No. 1312FT, Batch 03, was given to rats (Fischer 344) and mice (B6C3F1) via gavage (125 total doses, 5 days/week) for a period of 6 months at 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg body weight. After termination of 6-month exposure, rats and mice (treated and control of both sexes) were kept for 23 and 24 additional months respectively for lifetime observation.

MAJOR FINDINGS AND PROPOSED COURSE: The treatment (1.0 mg or higher dose levels) shortened the survival time in male rats. A significantly high incidence of atypical hepatocellular foci, neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas were observed in exposed rats (Tables 1 and 2). The incidence of hepatocellular carcinomas was also increased in both male (95%) and female (88%) mice (highest dose level) compared with control male (48%) and female (0%) mice (Table 3). The incidence of hepatic neoplasms appeared to be dose dependent in rats and mice. Liver tumors were observed only in those groups of animals to which PBB was given in doses sufficient to induce readily observable hepatic toxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fire retardant, Firemaster FF-1, a mixture of polybrominated biphenyls, was responsible for widespread environmental contamination, animal loss and possibly human illness in Michigan during 1973-1974. Long term effects in animals and possibly humans are still apparent because of the extremely long biological half-life and persistance in the environment. Because of this, it is important to evaluate in depth the long term toxicologic effects of the compound in laboratory animals in order to project the possible effects in man.

PUBLICATIONS

Gupta, B.N., McConnell, E.E., Harris, M.W., and Moore, J.A.: Polybrominated biphenyl toxicosis in the rat and mouse. Toxicol. Appl. Pharmacol. 57: 99-118, 1981.

TABLE 1

Incidence (%) of Microscopic Lesions in the Liver of Male Rats After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Dose mg/kg	Atypical/Foci	Neoplastic Nodules	Hepatocellular Carcinoma	Bile Duct Hyperplasia	Cholangio-carcinoma
0	3(1/33)*	0(0/33)	0(0/33)	24(8/33)	0(0/33)
0.1	8(8/39)	0(0/39)	5(2/39)	23(9/39)	0(0/39)
0.3	30(12/40)b	2(1/40)	0(0/40)	25(10/40)	0(0/40)
1.0	35(11/31)b	13(4/31)a	3(1/33)	42(13/31)	0(0/31)
3.0	39(13/33)b	12(4/33)	21(7/33)b	42(14/33)	0(0/33)
10.0	39(12/31)b	3(1/31)	23(7/31)b	29(9/31)	6(2/31)a
Dose Response	P<.01	NS	P<.01	NS	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.05) increased compared with control.
b Significantly (P<.01) increased compared with control.

TABLE 2

Incidence (%) of Microscopic Lesions in the Liver of Female Rats After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Dose mg/kg	Atypical/Foci	Neoplastic Nodules	Hepatocellular Carcinoma	Bile Duct Hyperplasia	Cholangio-carcinoma
0	0(0/20)*	0(0/20)	0(0/20)	10(2/20)	0(0/20)
0.1	0(0/21)	10(2/21)	0(0/21)	0(0/21)	0(0/21)
0.3	5(1/21)	0(0/21)	0(0/21)	0(0/21)	0(0/21)
1.0	18(2/11)	18(2/11)[a]	0(0/11)	9(1/11)	0(0/11)
3.0	21(4/19)	26(5/19)[a]	16(3/19)	21(4/19)	0(0/19)
10.0	40(8/20)[a]	40(8/20)[a]	35(7/20)[a]	35(7/20)	35(7/20)[a]
Dose Response	P<.01	P<.01	P<.01	P<.01	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.01) increased compared with control.

TABLE 3

Incidence (%) of Microscopic Lesions in the Liver of Male and Female Mice After a 6-Month Exposure to PBB and Examined During Lifetime Observation Phase

Sex	Dose (mg/kg)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Metastasis to Lung
Male	0	8(2/25)*	48(12/25)	16(4/25)
	0.1	4(1/27)	30(8/27)	0(0/27)
	0.3	17(4/24)	33(8/24)	8(2/24)
	1.0	8(2/25)	48(12/25)	8(2/25)
	3.0	9(2/23)	65(15/23)	13(3/23)
	10.0	5(1/22)	95(21/22)[b]	18(4/22)
	Dose Response	NS	P<.01	P=.03
Female	0	0(0/13)	0(0/13)	0(0/19)
	0.1	11(2/19)	0(0/19)	0(0/19)
	0.3	0(0/15)	13(2/15)	0(0/15)
	1.0	9(1/11)	18(2/11)	9(1/11)
	3.0	6(1/17)	18(3/17)	6(1/17)
	10.0	12(1/8)	88(7/8)[b]	38(3/8)[a]
	Dose Response	NS	P<.01	P<.01

* Data in parenthesis indicates number positive/number examined.
a Significantly (P<.05) increased compared with controls.
b Significantly (P<.01) increased compared with controls.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21009-01 STB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Reproductive Effects in Males Exposed to Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Lamb	Head, FRG	STB/TRTP	NIEHS
Others:	J. A. Moore	Deputy Director, NTP and Chief, EBB	STB/TRTP	NIEHS
	E. E. McConnell	Chief	CPB/TRTP	NIEHS
	J. K. Haseman	Research Statistician	BB	NIEHS
	W. M. Kluwe	Pharmacologist	CTEB/TRTP	NIEHS
	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

Pathology Branch (PB) Data Management and Analysis (DMA)
Lab. of Reproductive & Devleopmental Toxicology (LRDT)
Carcinogenic and Toxicologic Evaluation (CTE)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Fertility and Reproduction Group

INSTITUTE AND LOCATION

NIEHS; NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various environmental and industrial chemicals can disturb male reproductive function. The objective of these studies is to enhance our understanding of that toxic potential, and to further elucidate the mechanism of action in chemicals found to be toxic. Chemicals which are active as chemosterilants in males, such as kepone and dibromochloropropane, are used in various test systems. In addition to mechanistic studies, chemicals of unknown activity, such as the phenoxy herbicides and TCDD, have also been studied. Endpoints of toxicity include the assessment of spermatogenesis, sperm morphology, hormone levels and specialized chromosomal or genetic assays. Studies have also involved the assessment of progeny survival and development of treated males. These studies are expected to yield valuable data on chemical toxicity in males, as well as improve the sensitivity and accuracy of future testing systems.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: In addition to histological evaluation of testes and accessory sex organs, these studies involve assessment of sperm head morphology from the cauda epididymis and vas deferens. Chromosomal studies have been undertaken by studying sister chromatid exchanges in bone marrow and spermatogenic cells. Probes have also been prepared to study isolated portions of the DNA. The treated males have been studied by fertility and mating experiments and hormone patterns were studied in treated and control animals. Also, detailed teratologic evaluation of offspring have been used as an indicator of the potential for effects on subsequent generations.

MAJOR FINDINGS AND PROPOSED COURSE: Extensive studies have been completed on the effects of chlorinated phenoxy acids mixed with TCDD on the fertility of male mice and the incidence of congenital malformations in their progeny. A wide spectrum of fertility and genetic endpoints were used in those studies and no correlation could be identified between the chemical exposures and impaired reproductive function.

Subsequent studies are in progress which further investigate male germ cell toxicity as it relates to fertility and offspring development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The important potential of chemicals to alter fertility and reproductive function is just beginning to receive appropriate attention. Studies are anticipated or are in progress using known mutagens and/or chemosterilants which will expand our knowledge of these chemicals' toxic mechanisms. Such information will allow us to develop more predictive test systems in this field.

PUBLICATIONS

Lamb, J. C. IV, Marks, T. A., McConnell, E. E., Abeywickrama, K. and Moore, J. A.: Toxicity of chlorinated phenoxy acids in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6 male mice, J. Tox. Environ. Hlth., in press, 1981.

Lamb, J. C. IV, Marks, T. A., Gladen, B. C., Allen, J. W. and Moore, J. A.: Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2,3,7,8-tetrachlorodibenzo-p-dioxin, J. Tox. Environ. Hlth., in press, 1981.

Lamb, J. C. IV, Marks, T. A., Haseman, J. K. and Moore, J. A.: Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-bioxin, J. Tox. Environ. Hlth., in press, 1981.

Author: Lamb, J. C. IV, Moore, J. A. and Marks, T. A. Evaluation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in C57BL/6 mice: Reproduction and fertility in treated male mice and evaluation of congenital malformations in their offspring. Department of Health and Human Services (DHHS), National Toxicology Program, Technical Report No. NTP-80-44, 1980, 57 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 21010-01 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Kepone Toxicity as a Model for An Environmental Chemicals' Influence on Female Reproduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Lamb, IV	Head, FRG	STB/TRTP	NIEHS
Other:	J. A. Moore	Deputy Director, NTP	STB/TRTP	NIEHS
	E. E. McConnell	Chief, PB	CPB/TRTP	NIEHS
	K. S. Korach	Research Endocrinologist	LRDT	NIEHS

COOPERATING UNITS (if any)

Pathology Branch (PB)
Lab. for Reproductive and Developmental Toxicology (LRDT)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Fertility and Reproduction Group

INSTITUTE AND LOCATION
NIEHS; NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.55	0.95

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of these studies is to evaluate the potential of environmental chemicals to affect female reproductive function. These studies compare toxic effects at high and low levels of exposure. As a model compound for these experiments, we are studying the effects of Kepone on female reproductive function. The toxicity of these compounds is evaluated using a broad spectrum of toxic indicators. Since these effects, and the effects of other environmental compounds, may be mediated through their estrogenic or other hormonal activity, we have established a number of criteria which indicate hormone activity. Uterine, ovarian and pituitary function are studied in morphological and endocrinological studies after Kepone exposure. Fertility and reproduction are also evaluated in treated females and in offspring exposed perinatally to Kepone. Morphological studies include light and scanning electron microscopy, hormone and xenobiotic autoradiography, and histochemistry. Biochemical studies include hormone radioimmunoassay and hormone receptor assays. It is anticipated that these studies will help establish the mechanism of reproductive toxicity of compounds such as Kepone. Such data should also lead to more efficient and accurate testing systems in reproductive toxicology.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Initial studies on the reproductive toxicity of chemicals have involved the assessment of fertility. These investigations included the long-term chemical exposure of female rats and mating to untreated males. The fertility of the perinatally exposed offspring was also studied.

In circumstances where an effect on fertility in the exposed female or their offspring has been detected, such as with Kepone, various specialized morphological and biochemical studies are undertaken. Morphological studies include light microscopy and scanning electron microscopy. These studies are performed such that a single tissue may be studied by both methods to enhance the interpretation of effects. Various histochemical and immunocytochemical studies are also undertaken to localize specific enzymes or peptides in the tissue. The autoradiographic subcellular localization of steroid hormones has been studied. The localization of ^{3}H-estradiol in the uterus is of special interest, since Kepone may act through the same receptors as estradiol and the localization of estradiol will be compared to radiolabeled-Kepone localization. These morphological studies of hormone-receptor action are being correlated with biochemical hormone receptor studies on estradiol and Kepone.

MAJOR FINDINGS AND PROPOSED COURSE: Kepone does act as an antifertility agent and at relatively high concentrations, both male and female reproduction are impaired. However, as levels of Kepone are reduced, the female appears to be more sensitive to Kepone's reproductive toxicity. Additional studies have indicated that perinatal exposure to Kepone may be especially toxic to the female offspring at levels low enough to be apparently non-toxic to the mothers or the male offspring. Special attention is being given to these perinatal effects and the endocrinological status of these female offspring is being investigated. Future studies will employ the methods described and are expected to yield a better understanding of the manner by which Kepone, and other environmental chemicals, may alter reproductive functions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30042-06 STB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Contaminants on Cardiac Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	STB/TRTP NIEHS
	M. P. Moorman	Biomedical Engineer	LPFT NIEHS
	R. Sloane	Biological Laboratory Technician	STB/TRTP NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.40	0.25	0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward the detection of functional changes in the cardiovascular system associated with compounds of environmental significance to which toxicologically significant exposure would be expected by inhalation. Allylamine has been reported to produce myocardial necrosis in experimental animals. We have given rabbits allylamine in the drinking water in concentrations less than those reported to produce myocardial necrosis detectable by light microscopy. Myocardial function subsequently assessed in vitro indicates that measurable functional impairment may precede the appearance of frank, structural damage. A possible sparing effect of concurrent treatment of rabbits with allylamine and aminoguanidine is under investigation.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The effects of environmental chemicals on the heart are studied using isolated, perfused (Langedorff) rabbit hearts and other isolated heart muscle preparations.

MAJOR FINDINGS AND PROPOSED COURSE: Contractility is reduced in the hearts of rabbits exposed to 5 mM allylamine in the drinking water for 3 weeks prior to testing. The hearts are substantially depleted of sympathetic neurotransmitter by prior treatment with 6-OH-dopamine which effectively rules out a mechanism mediated through the modulation of endogenous sympathetic activity. Since diamine oxidase is capable of converting allylamine to acrolein, experiments are underway to attempt to modify allylamine cardiotoxicity through the inhibition of diamine oxidase with aminoguanidine. It should be noted that a paper has been published recently in which the authors reported an inability to detect acrolein in the hearts of animals poisoned with allylamine, leading them to rule out this possible mechanism of toxic action. The extreme reactivity of acrolein would suggest the possibility that it yet may be shown to be involved, but may be present in concentrations below easily detected limits, or may represent an extremely short-lived, reactive intermediate.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Allylamine represents one of a growing list of chemicals and drugs that have been demonstrated to produce chemical injury to the heart. Evaluation of this chemical as well as others in the future represents a continuing effort to establish a research base in cardiovascular toxicology at NIEHS.

PUBLICATIONS

Van Stee, E.W.: Myocardial Toxicity. In Witschi, H (Ed.): The Scientific Basis of Toxicity Assessment. Amsterdam, Elsevier/North-Holland, 1980, pp. 167-182.

Van Stee, E.W. (Ed.): Cardiovascular Toxicology. New York, Raven Press, 1981.

Van Stee, E.W.: Cardiovascular Toxicology: Foundations and Scope. In Van Stee, E.W. (Ed.): Cardiovascular Toxicology. New York, Raven Press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES30044-05 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Toxicology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Van Stee	Physiologist	STB/TRTP NIEHS
OTHER:	M.P. Moorman	Biomedical Engineer	LPFT NIEHS
	J.E. Simmons	Graduate Student	STB/TRTP NIEHS
	R.A. Sloane	Biological Laboratory Technician	STB/TRTP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.1	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Rats were exposed by inhalation to 7 different concentration profiles of carbon tetrachloride. Each profile had a total concentration times time equal to 4500 ppm-hr. Some of the profiles could be distinguished from others based on histopathological changes in the livers. The toxic response was not consistently related to number of, duration of, or interval between pulses.
2. and 3. Studies are underway to determine the mechanism of the acceleration of atherosclerosis by carbon disulfide.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: 1. Male, CD rats were exposed in groups of 12, to 7 different exposure profiles of carbon tetrachloride (CCl4) in which the maximum concentration was 1500 ppm. The exposures were conducted in a computer-assisted inhalation facility in dynamic flow-through chambers of a capacity of 400 liters. The flow rate was 100 liters/min. The exposures consisted of sequences of approximately rectangular pulses of varying duration and interval. The total concentration-times-time (CxT) for each profile was 4500 ppm/hr. The rats were exposed daily for 4 consecutive days and killed on the 5th day. Sections of livers from each of 3 rats selected at random from each exposure group of 12 were examined microscopically. Features of necrosis and vacuolation were recorded as either present or absent and severity was quantified on a scale of 0 through 4. Experiments were replicated at least 6 times. Ranks representing severity were transformed to normal scores. All data were analyzed using the analysis of variance with Duncan's New Multiple Range Test, and multiple linear regression analysis. 2. Male CD rats and NZW rabbits were exposed to carbon disulfide (CS2), 300 ppm, 6 hr/da, 5 da/wk for 12 wk in the facility described in #1. The animals were weighed and thyroid function tests were performed every 2 wks. 125-I uptake was determined by gamma counting and serum activities of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) were done by radioimmunoassay (RIA). Data were analyzed using the analysis of variance and regression analysis. 3. Twenty-seven male, NZW rabbits were divided into groups of three and exposed to all possible combinations of 0, 1, or 2% dietary cholesterol, and 0, 100, or 300 ppm of CS2, respectively, for 12 wk. Changes in serum and aortic lipids were analyzed as indexes of the atherogenic process. Fat-stained, frozen sections of arteries, and sections stained with H&E following standard fixation were examined microscopically. Data were analyzed using the analysis of variance and regression analysis.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Some of the CCl4 exposure profiles could be distinguished from others by the histopathological changes that were observed in the liver. Hepatotoxicity was not significantly related to number of leading edges (number of pulses), duration of pulses, or the interval between the pulses. Either we haven't been able to extract the information or we don't have enough information to decide what characteristics of the profiles are responsible for the differential responses. A better model than ours could be chosen for the purpose of investigating the dependency of an expression of toxicity on exposure profile. The model should exhibit a readily quantifiable dose-response, and interpretation of the endpoints should not be confused by complicated pharmacokinetics. A chemical not requiring activation and acting as a primary pulmonary toxin might be suitable. 2. Data from this experiment were not available at the time of the writing of this report. If a depression of thyroid activity can be correlated with an acceleration of atherogenesis in this and other experiments, future experiments will be conducted in which animals will be exposed to an atherogenic diet and CS2, and fed thyroid hormones to see if thyroid replacement therapy mitigates this effect of exposure to CS2. 3. Data from this experiment were not available at the time of the writing of this report. If a fraction of the atherogenic effect of exposure to CS2 is found not to be simply a secondary response to hyperlipidemia experiments will be conducted to examine the possibility that CS2 induces intimal injury that could trigger the atherogenic cascade described by the "injury hypothesis" of

atherogenesis. 4. If the resources become available CS2 will be screened using the Strain A/J mouse, carcinogen bioassay system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1. We have concluded that under certain circumstances the shape of the concentration profile to which animals are exposed may affect the outcome of experiments in inhalation toxicology. The time-weighted average concentration may not always represent the best model of time-varying, real-life conditions. Few comparisons of different exposure profiles with equal time-weighted concentrations have been reported in the past because of the technical difficulty in simulating the conditions in the laboratory. Computer-assisted operation of inhalation facilities eliminates this impediment to research. Exposure profiles monitored in the real world may now be simulated easily and reproducibly thus paving the way for ruling in or out the significance of this generally ignored variable. 2. Observers estimate that between 20,000 and 500,000 (depending on who is doing the reporting) Americans are exposed to CS2. CS2 has a multiplicity of toxic effects and, despite decades of research, many areas remain relatively unexplored. The National Toxicology Program has selected CS2 for further study, and the results of our efforts are expected to clarify the matter of whether or not the depression of thyroid activity that is induced by exposure to CS2 contributes to the acceleration of the development of atherosclerosis that also accompanies long-term, low-level exposure to CS2. If hypothyroidism turns out to be of clinical significance, and exposure to CS2 cannot be avoided, results of our studies may show that it could be possible to mitigate the effects by the appropriate therapy.

PUBLICATIONS

Van Stee, E.W., Wynns, P.C., and Moorman, M.P.: Distribution and disposition of morpholine in the rabbit. Toxicology, 1981 (in press).

Lam, H.F., Takezawa, J., Gupta, B.N., and Van Stee, E.W.: A comparison of the effects of paraquat and diquat on lung compliance, lung volumes and single breath diffusing capacity in the rat. Toxicology 18: 111-123, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30045-04 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Development of an Automatic Small Animal Inhalation Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. P. Moorman	Biomedical Engineer	LPFT NIEHS
	E. W. Van Stee	Physiologist	STB/TRTP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves the design and implementation of microcomputer based systems to monitor and control gas concentrations in small animal inhalation exposure facilities. Included in this effort are the development of hardware, software and operational procedures necessary for the complete utilization of the systems.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Data acquisition and feedback control theory have been used to develop a microcomputer-based sampled data control system capable of regulating 9 chambers on a time multiplex basis. A second version of the system is being developed for deployment in the contractor-operated inhalation facility of NIEHS based on identical theoretical considerations but employing a network of computers to monitor and control 12 chambers designed for gas inhalation studies in small laboratory animals.

MAJOR FINDINGS AND PROPOSED COURSE: Since this system must regulate gases generated from compounds with different physical properties, it has been necessary to design a control system capable of measuring certain characteristics of each generating system and adapting the control equations to optimize responses for each particular compound.

Because equipment calibration is a significant factor in system performance statistical procedures have been implemented to evaluate daily calibrations and long term system performance.

In order to define better the exposure environment, temperature and humidity are monitored and animal excrement actively removed during the course of the exposure.

The data conversion equation on which machine control is based is derived from 6-point calibration curves to which polynomials are fitted by the method of least squares. The calibration scheme has been designed to maintain rigorous control of both chamber technician performance of the calibration routines and the machine operation of the system. Independent calibration checks are by GC-MS analysis.

Detailed documentation of system design and operation is in preparation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. Machine control of inhalation chambers can produce an accurately controlled and documented exposure resulting in reduced technical error.

2. Statistical evaluation of periodic calibrations can quantify monitoring accuracy and identify many calibration errors.

3. Documentation of the exposure conditions and profiles can be summarized in a compact and meaningful format eliminating the need for hand analysis of chamber output data.

4. More complicated exposures such as time varying concentrations of multiple compounds are possible with machine control more than human operators.

PUBLICATIONS

Van Stee, E.W., and Moorman, M.P.: Monitoring for Temperature, Humidity and Concentration. In Drew, R.T. (Ed.): Proceedings on a Symposium of Inhalation Chamber Technology. New York, Brookhaven National Laboratories, 1981, pp. 12/1-9.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30084-04 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Halogenated Biphenyls and Related Hydrocarbons on Drug-Metabolizing Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Goldstein	Pharmacologist	STB/TRTP	NIEHS
OTHER:	K. K. Kohli	Visiting Associate	STB/TRTP	NIEHS
	P. Linko	Chemist	STB/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:
2.0	1.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the structure-activity requirements for induction of hepatic mixed-function oxidases by polychlorinated biphenyls (PCBs) and other related halogenated aromatics, and to identify the biochemical changes which occur when rats are exposed to those compounds. Present work includes purification and identification of subspecies of cytochrome P-450 induced by PCBs, phenobarbital, 3-methylcholanthrene and other halogenated aromatics such as chlorinated benzenes. Present work has identified the induction of at least 2 subspecies of cytochrome P-450 by 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) with molecular weights (mws) of 55,000 and 52,000 and an additional 52,000 mw subspecies by 2,3,5,2',3',5'-HCB. These 3 subspecies differed spectrally and catalytically. The present objectives of this work are to: 1) identify the subspecies of cytochrome P-450 induced by these compounds utilizing purification, SDS-polyacrylamide gel electrophoresis, reconstitution, and antibodies to the purified forms.

PHS-6040
(Rev. 2-81)

MAJOR FINDINGS: 1) Continuing structure-activity studies for induction of cytochrome P-450(s) by PCBs have shown that PCBs can be divided into 2 classes of inducers in the rat. a) Biphenyls with halogens in at least four of the 3,4,3',4'-positions are 3-methylcholanthrene (3-MC) type inducers. b) Biphenyls with ortho halogens are phenobarbital type inducers if highly halogenated. c) Biphenyls with few halogens are weak or inactive. Some nonsymmetrical biphenyls are also inactive despite a high degree of chlorination.

2) Rats exposed to hexachlorobenzene (HCB) shown have been pattern of induction intermediate between that of phenobarbital and 3-MC. Purification of HCB using a charcoal column to remove possible planar impurities did not alter the inductive properties of HCB. A number of other di-, tri-, tetra- and pentachlorobenzenes were phenobarbital-type inducers. SDS-PAGE indicated that HCB induces a protein band identical in molecular weight (mw) to cytochrome P-450b, and a second band identical to a previously unidentified cytochrome found in 3,4,5, 3',4',5'-PCB treated microsomes. Cytochrome P-448 did not appear to be induced.

3) DEAE chromatography of solubilized microsomes from control, 2,3,5,2',3',5'-PCB and 3,4,5,3',4',5'-PCB treated rats have been compared. Three distinctly different cytochromes were found to be induced. 2,3,5,2',3',5'-HCB induces a cytochrome which has a molecular weight of 52,000 and on reconstitution metabolizes benzphetamine. 3,4,5,3',4',5'-HCB induces a cytochrome with a molecular weight of 55,500 which metabolizes ethoxyresorufin. 3,4,5,3',4',5'-HCB also induces a second cytochrome with a molecular weight of 52,000 which differs spectrally from the above cytochromes and does not metabolize either ethoxyresorufin or benzphetamine when reconstituted with lipid and NADPH cytochrome c reductase. This new cytochrome has been further purified by chromatographic techniques to 13.6 nmol/mg of protein. Its CO-reduced difference spectrum has a maximum at 448 nm, and its subunit molecular weight is 52,000. Cytochrome P-450b from phenobarbital-treated animals has also been purified.

PROPOSED COURSE: Antibody to the cytochrome isolated from 3,4,5,3',4',5'-PCB treated rats will be raised in rabbits. Cytochrome P-450 c from 3-methylcholanthrene treated rats is presently being purified. The new cytochrome will be compared with cytochromes P-450b and P-450c using antibody, peptide maps, and reconstitution studies with a variety of substrates. Antibody will be used to identify these cytochromes in whole microsomes. Collaborative studies on production of mutagenic metabolites by these cytochromes will be initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PCBs and hexachlorobenzene are environmental contaminants. There has been considerable human exposure to PCBs and polybrominated biphenyls (PBBs). Initial experiments analyzed the structure-activity relationships of PCBs and PBBs. Toxicity appears to correlate with structure-activity relationships. Those compounds which induce P-448 are the most toxic and appear to produce a totally different biological response than those which induce cytochrome P-450. The structure-activity relationships for hexachlorobenzene suggest that only this chlorobenzene isomer will be toxic. Present investigations will hopefully yield information on interactions with other chemicals metabolized by the liver to mutagens and carcinogens.

PUBLICATIONS

Kohli, K. K., Linko, P., and Goldstein, J. A.: Multiple forms of solubilized and partially resolved cytochrome P-450 from rats induced by 2,3,5,2',3',5'- and 3,4,5,3',4',5'-hexachlorobiphenyls. Biochem. Biophys. Res. Commun., In press, 1981.

Goldstein, J. A., Linko, P., McKinney, J. D., and Albro, P. W.: Marked differences in the inductive effects of two symmetrical hexachlorobiphenysl and the corresponding unsymmetrical isomer on hepatic monooxygenases. Biochem. Pharmacol., In press, 1981.

Goldstein, J. A.: Structure-activity relationships for the biochemical effects of halogenated aromatic hydrocarbons and the relationship to toxicity. In: R. Kimbrough (Ed.), Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, Elsevier, North Holland, pp. 151-191 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30098-03 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Oncogenic Potential of NO_2 Inhalation in Small Animals Exposed to Heterocyclic Amines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Van Stee	Physiologist	STB/TRTP NIEHS
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	G.A. Boorman	Veterinary Pathologist	CPB/TRTP NIEHS
	J.E. Simmons	Graduate Student	STB/TRTP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Inhalation Toxicology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

CD-1 mice were exposed to NO2 and morpholine, NO2 alone, morpholine alone, or neither, intermittently for 30 wk. The incidence of pulmonary adenomas detected during the following 2 yr was significantly higher in the combination group than in the other treatment groups or controls. Work is continuing in an attempt to determine the mechanism of the in vivo formation of a putative carcinogen, possibly N-nitrosomorpholine. Strain A/J mice were found to develop pulmonary adenomas in numbers that were a function of dose of urethane and time after exposure. Doses ranged from 250 to 1000 mg/kg. Lungs were examined 4, 5 and 6 months after injection. Urethane is used as a positive control carcinogen.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: We exposed male CD-1 mice (Charles River) and CDF rats in groups of 35 to 1-2 ppm of NO2 for 6 hr/da, 5 da/wk for 30 wk and to 0.1% morpholine in the drinking water in all combinations for 30 wk. Controls received plain water and conditioned air. The animals were allowed to live out their natural lifespans and died throughout the 2nd and 3rd years postexposure. The animals were examined grossly and histopathologically for the presence of lesions related to the exposures. 2. Mice were exposed to 50 ppm of NO2 or (15-)NO2 or plain air for 6 hr, with or without concurrent exposure to 1 g/kg of morpholine by gavage. Pools of trachea-lung blocks, stomach and gastric contents, and whole mice were analyzed for the presence of N-nitrosomorpholine, (15-)N-nitrosomorpholine, N-nitromorpholine, and (15-)N-nitromorpholine by gas chromatography (thermal energy analysis) or gas chromatography-mass spectrometry, as appropriate. 3. 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, and 1000 mg/kg of body weight. Groups representing each dosage were killed at 4, 5 and 6 months postinjection, respectively. Lungs were fixed with Tellyesniczky's solution and examined 24 hr later for the presence of pulmonary adenomas by 3 technicians working independently. 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 post-exposure.

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 (15-)NO2-morpholine study of the formation of N-nitrosomorpholine and N-nitromorpholine were not available at the time that this report was written. 3. The data from the urethane study are contained in the following table. The numbers in the body of the table represent surface tumors per mouse (n=20 per group). The numbers in parenthesis were obtained by Dr. H.P. Witschi at the Oak Ridge National Laboratory in a separate, but otherwise identical experiment conducted on male, Strain A/J mice that had been obtained from the same source as ours.

	Urethane in 0.9% NaCl, mg/kg IP			
	Control	250	500	1000
4 months	0.3	4.9 (3.9)	8.4 (10.6)	17.6 (23.2)
5 months	0.3	4.2	11.2	21.1
6 months	0.3	5.9 (6.5)	11.7 (10.6)	25.8 (26.7)

The data were analyzed using the analysis of variance. The pooled standard deviation was 3.68. Minor differences among the tumor counts reported by the different technicians were not statistically significant. Our data were in

remarkable agreement with those obtained by Dr. Witschi and we interpreted this to be verification of the validity of the bioassay in our hands. These data will be compared with data obtained from positive (urethane) controls that are a part of the companion study being done on contract (see Northrop Services, Inc., NO1-ES-79-0009).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND 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 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. Results of experiments with urethane in the Strain A/J mouse bioassay system have justified a trial of the bioassay in our studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30099-02 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of 2,3,7,8-Tetrachlorodibenzodioxin and Polychlorinated Biphenyls on Arachidonic Acid Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Goldstein	Pharmacologist	TRTP NIEHS
OTHER:	K. Kohli	Visiting Associate	TRTP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
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SECTION
Biochemical Toxicology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 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 (200 words or less - underline keywords)

The purpose of this work was to explore the possibility of a relationship between the effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) on prostaglandin synthesis. This work examines the effects of TCDD on conversion of arachidonic acid to prostaglandins in rabbit kidney medulla and rabbit liver microsomes.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The effects of TCDD on microsomal synthesis of prostaglandins from [^{14}C] arachidonic acid in rabbit liver and kidney medulla were examined 24 and 72 hr after TCDD administration. A hepatotoxic dose of TCDD (30 µg/kg) did not affect prostaglandin synthetase activity of rabbit liver or kidney medulla microsomes at either time point. Furthermore, TCDD did not affect the pattern of prostaglandins synthesized. In contrast, TCDD increased cytochrome P-450 of rabbit liver microsomes 2-fold and that of kidney medulla 3.

PORPOSED COURSE: As the result of discussions with other investigators in this field, this study will be extended collaboratively to the lung (rat). Arachidonic acid release from membranes will also be examined in culture.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There has been considerable interest in human exposure to TCDD and PCBs. It is generally believed that TCDD produces toxicity by interacting with a cytosolic receptor and the subsequent induction or repression of enzyme(s) which result in TCDD toxicity. It is of interest to identify the biochemical event(s) which lead to toxicity.

PUBLICATIONS

Kohli, K. K. and Goldstein, J. A.: Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic and renal prostaglanding synthetase. Life Sciences, In press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30106-02 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Development and Validation of Immunology and Host Resistance Assays to Detect Chemical Induced Immunotoxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G.A. Boorman	Veterinary Pathologist	CPB/TRTP NIEHS
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	L.D. Lawson	Biological Laboratory Technician	STB/TRTP NIEHS
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Immunobiology

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 (200 words or less - underline keywords)

The objectives of this project are to clarify differences among several methods for measuring the same immunological parameter(s) and to find the most sensitive and reproducible test method(s) for assessing immunotoxicity and altered host resistance in order to define a testing battery. We are presently developing, refining and applying methods for measuring delayed hypersensitivity as well as T-lymphocytes, B-lymphocytes, macrophages and bone marrow cell functions to define chemical-induced immunotoxicity and altered host resistance using suspect or known immunotoxicants. Included in this effort are the validation and confirmation of the sensitivity of the selected methodologies using environmental chemicals suspect of altering the immune system. These studies will hopefully allow correlations between changes in immunological parameters to provide prognosticators of alterations in host resistance to bacteria, viruses, parasites or transplantable tumor cells.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The assays that are under development or being validated to assess immunological dysfunction or altered host resistance following chemical exposure are listed in the testing panel described in Table 1. Major emphasis has been placed on assays that can be automated, routinized and miniaturized.

MAJOR FINDINGS AND PROPOSED COURSE: Studies involving the validation of the PYB6 tumor, Trichinella spiralis and Listeria monocytogenes challenge models have continued during the past year with excellent correlations found between altered host resistance and depression of T-cell and macrophage immunocompetence. A new artificial metastasis tumor model was developed using the B16F10 melanoma cell line for examining systemic and lung-specific immune surveillance following chemical exposure. In this model injected tumor cells produce tumor foci in the lung proportionate to the T-cell dysfunction induced. During the coming year, attempts will be made to validate this model following inhalation exposure to an environmental chemical. The bone marrow toxicity evaluation panel was expanded to include an Fe^{59} incorporation assay to detect alteration in erythropoiesis (red blood cell synthesis) following chemical exposure. This panel has now been validated and found capable for detecting chemical injury to pleuripotent stem cells (CFU-S), granulocyte and monocyte precursors (CFU-GM) and erythrocyte precursors. Capabilities in quantitating thymus-dependent lymphocyte subpopulations have been expanded with the addition of Lyt reagents for quantitating murine helper (Lyt 1) and suppressor cells (Lyt 2,3). With these reagents loss of T-cell subsets following chemical exposure should be detectable.

Capabilities for detecting altered macrophage function have been expanded through the development of an in vitro bactericidal assay using Staphylococcus aureus and an evaluation panel for macrophage lysosomal enzyme. The macrophage cytotoxicity assay was deleted from the panel after it was found that an assay employing cytostasis of leukemia target cells had a greater level of sensitivity for detecting activation.

Studies with the non-steroidal estrogenic chemical diethylstilbestrol (DES) were continued focusing on the mechanism by which macrophages activated by DES exposure fail to kill bacteria or tumor cells in vivo. These studies required the development of a panel of assays for lysosomal enzymes. Preliminary results suggest that DES exposure results in activated macrophages which have unelevated levels of certain enzymes believed essential for lysis of bacteria. These studies will continue during the coming year.

Immunological studies were initiated with the polycyclic aromatic hydrocarbon carcinogen benzo(a)pyrene (B[a]P). B(a)P is formed by incomplete combustion of fossil fuels and as a by-product of the coal conversion process. The non-carcinogenic congener B(e)P was evaluated in parallel. B(a)P exposed mice demonstrated severely depressed antibody responses, moderately depressed lymphoproliferative responses to mitogens and a reduced number of bone marrow macrophage-granulocyte progenitors. Macrophage function and host resistance to Listeria monocytogenes and syngeneic tumor cells was unaffected. The toxicity of B(a)P appeared selective for B-cells involved in antibody production. This lesion might explain our failure to observe effects in our host resistance models which

primarily involve cell-mediated immunity. The type of immune dysfunction observed is probably not sufficient to play a significant role in the carcinogenicity of this chemical. The failure of the non-carcinogenic congener B(e)P to induce immune dysfunction may indicate some yet undefined causal relationship between the structural requirements for immunosuppressive and carcinogenic potential.

These studies will continue using other carcinogen/non-carcinogen PAH pairs to mo re firmly establish these structural relationships. Since it is now suggested that many environmental chemicals may act as promoters of carcinogenesis rather than be carcinogenic, the potent tumor promoter phorbol mystric acetate (PMA) was evaluated for immunological effect. Exposure of B6C3F1 mice to PMA resulted in severly impaired cell-mediated immunity, depression of T-cell numbers and enhanced susceptibility to challenge with both the PYB6 (sarcoma) and B16 melanoma cell lines but not bacterial challenge. Concomitant with the selectively altered host resistance to tumors was a loss of natural killer cell activity and a T-cell function believed responsible for immune surveillance against tumor cells. Other isomeric forms of the phorbol esters which have less levels of promoter activity will be studied during the coming year in our continued validation and development of sensitive methods for detecting immunotoxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A correlation has been clearly established between the administration of chemical immunosuppressants and an increased incidence of infectious diseases and neoplasia. The evidence for increased bacterial, viral, fungal and parasitic diseases in patients on chronic immunosuppressive chemicals has been well documented by Allen (Infection complicating neoplastic disease and cytotoxic therapy. In: Infection and the Compromised Host, 1976). Likewise, McKhann (Transplantation 8:209, 1971) observed that the incidence of cancer in renal transplant recipients on prolonged immunosuppressive chemotherapy was 4.6-61 times higher than in the general population.

Studies in laboratory animals also have supported these clinical observations and demonstrated an enhanced incidence of UV-induced or benzopyrene-induced cancer in mice treated with immunosuppressive agents. The mechanistic relationship between altered host resistance and immune dysfunction is complex, poorly defined and of extreme importance. Chemicals of environmental concern have been recently shown to induce immunosuppression as evident by depressed antibody mediated immunity, cell-mediated immunity or MØ dysfunction in rodents following sublethal exposure. Some of the chemicals which induce immunologic effects in rodents include 2,3,7,8-tetrachlorodibenzo-p-dioxin, polychlorinated biphenyls, polybrominated biphenyls, gallic acid, DES, BP, hexachlorobenzene, pentachlorophenol, certain organo- and heavy metals. Some studies have indicated that exposure to certain chemicals can alter resistance to bacteria, viruses, parasites and transplantable tumor cells. Of major concern is the correlation of these immunologic findings with altered host susceptibility and the extrapolation of these chemically induced immunobiologic effects to humans.

PUBLICATIONS

Luster, M.I., Dean, J.H., and Moore, J.A.: Evaluation of immune functions toxicology. In Hays, W. (Ed.): Methods in Toxicology. New York, Raven Press (in press).

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activation. In Hersh, E.M. (Ed.): Augmenting Agents in Cancer Therapy. New York, Raven Press, 1981, pp. 267-284.

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Luster, M.I., Boorman, G.A., Dean, J.H., Harris, M.W., Luebke, R.W., Thigpen, J.E., Padarathsingh, J.L., and Moore, J.A. Examination of bone marrow, immunological parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Int. J. Immunopharmacol. 2:201-310, 1980.

TABLE 1

Comprehensive Screening Panel for Defining Immune Alterations Currently Being Evaluated at NIEHS

Parameter	Procedure Performed
Pathotoxicology	Hematology Profile-hemoglobin, red blood cell count, white blood cell count, differential
	Clinical Chemistries-CPK, αHBDH, SGTP, BUN, creatinine, acid and alkaline phosphatase, LDH, cholinesterase
	Serum Proteins-albumin, globulin, A/G, total proteins
	Weights-body, spleen, thymus, liver, kidney
	Histology-liver, thymus, adrenal, lung, kidney, heart, spleen
Host Resistance	Tumor Assays-tumor cell challenge TD_{10-20} and radiometric tumor mass
	Listeria monocytogenes LD_{10-20} challenge
	Endotoxin hypersensitivity-LD_{10-20}
	Expulsion of Trichinella spiralis
Delayed Hypersensitivity	Radiometric assay with T-cell dependent antigen
Lymphocyte Proliferation	One-way mixed leukocyte culture
	Mitogens-PHA, Con A, LPS

TABLE 1 CONTINUED

TABLE 1 CONTINUED

Humoral Immunity	Immunoglobulin levels (IgG, IgM, IgA)
	Antibody response to T-dependent (SRBC) and T-independent (LPS) antigens
Macrophage Function[1]	Resident peritoneal cell numbers and nonspecific esterase staining
	Phagocytosis
	Lysosomal enzymes-5'-nucleotidase, acid phosphatase, leucine amino peptidase
	Cytostasis of tumor target cells
	RES clearance using ^{125}I-triolein
Bone Marrow Colony Forming Units	CFU-S-multipotent, hematopoietic stem cells
	CFU-GM-granulocyte/macrophage progenitor
	Cellularity
	^{59}Iron incorporation in bone marrow and spleen

[1]Employs both resident peritoneal cells and pyran activated macrophages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35004-03 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Metabolism and Disposition of Halogenated Alkyl Phosphates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	STB/TRTP NIEHS
	Amin Nomeir	Visiting Fellow	STB/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:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tris (2,3-dibromopropyl) phosphate (Tris) and tris (1,3-dichloroisopropyl) phosphate (Fyrol FR-2) have been studied in the male rat following iv., oral and dermal administration. Each of these compounds is absorbed from the gastrointestinal tract. Following absorption or iv injection these compounds are rapidly metabolized and excreted. The major metabolites are dealkylation products which are excreted primarily in the urine with lesser amounts being excreted in the feces or metabolized to CO_2 and exhaled. In vitro studies have demonstrated that metabolism is mediated by both the microsomal mixed function oxidases and a soluble glutathione-S-transferase. A study of covalent binding to subcellular macromolecules has demonstrated that Tris has a greater affinity for DNA than does Fyrol and that this difference is most pronounced in the kidney.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The metabolism, distribution and excretion of two halogenated alkyl phosphates have been studied in the male rat following oral, dermal or iv administration. The compounds studied were tris (2,3-dibromopropyl) phosphate (Tris) and tris (1,3-dichloroisopropyl) phosphate (Fyrol FR-2). Each was radiolabeled with carbon-14. The metabolism of each compound was studied both in vivo and in vitro. Metabolites were isolated by extraction with organic solvents and purified by thin-layer and high-performance liquid chromatography. Identification of metabolites was achieved by cochromatography with chemically synthesized metabolites. Tissue distribution was studied by sampling at various time points and oxidizing the samples to $^{14}CO_2$ in a biological material oxidizer. Excretion was followed by collecting samples of urine, feces and exhaled CO_2. Quantitation of samples was by liquid scintillation counting.

MAJOR FINDINGS: 1) The distribution, metabolism and excretion of Tris and Fyrol FR-2 were similar. Both compounds are readily absorbed from the gastrointestinal tract, are also absorbed from the skin and distribution following absorption or iv injection was not affected by the route of administration.

2) The major mechanisms of metabolism involve dealkylations of the phosphate. The dealkylated products undergo various degrees of further metabolism and may be metabolized all the way to CO_2. In vitro studies have demonstrated that the dealkylations are mediated by both mixed-function oxidases and a soluble glutathone-S-transferase.

3) Tris and Fyrol-FR-2 have short biological half-lives and are excreted primarily in the urine with lesser amounts being eliminated in the feces or metabolized to CO_2 and exhaled. The half-lives of the parent compounds are a matter hours whereas the half-lives of the metabolites vary from approximately 2 to 4 days.

4) Trace amounts of each of these compounds are metabolized to reactive intermediates which alkylate subcellular macromolecules. The major differences relating to structure are observed in the alkylation of DNA in the kidney where treatment with Tris results in approximately 10 fold greater DNA alkylation than results from Fyrol treatment.

5) The studies indicate that a critical factor affecting the mutagenicity of these two compounds may be the structure of the haloalkyl side chains. The 2,3-dibromopropyl side chain of Tris is metabolized to a halogenated propene which may be a precursor of a reactive intermediate whereas no similar metabolite of Fyrol FR-2 was observed.

PROPOSED COURSE:

These studies will be completed, the results prepared for publication and this project will be terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITITUTE: The halogenated alkyl phosphates studied in this project have been or are currently used as fire-retardants in fabrics and flexible foam upholstery. Both Tris and Fyrol were at one time used in children's pajamas. Tris has been shown to be mutagenic in the Ames bioassay system and carcinogenic to laboratory animals; however, Fyrol is an order of magnitude less mutagenic in the Ames bioassay system and extensive testing has yet to show that Fyrol is carcinogenic to laboratory animals. Therefore, studies of these two compounds offer an opportunity to relate structure to activity in the mediation of a toxic effect by closely related compounds. The results of these studies will facilitate an understanding of the factors which result in toxicity and will provide information which should aid in the recognition of other potentially toxic compounds or facilitate the design of less toxic compounds which may be synthesized in the future.

PUBLICATIONS

Morales, N. M. and Matthews, H. B.: In vivo binding of the flame retardants tris(2,3-dibromopropyl) phosphate and tris (1,3-dichloro-2-propyl) phosphate to macromolecules of mouse liver, kidney and muscle. Bull. Environ. Contam. Toxicol. 25: 34-38, 1980.

Nomeir, A. A., Kato, S. and Matthews, H. B.: The metabolism and disposition of tris (1,3-dichloro-2-propyl) phosphate (Fyrol FR-2) in the rat. Toxicol. Appl. Pharmacol. 7: 181-191, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-08 STB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics of Chlorinated Xenobiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	STB/TRTP NIEHS
Other:	Daniel B. Tuey	Staff Fellow	BB NIEHS
	Gary M. Decad	Staff Fellow	STB/TRTP NIEHS
	Fatama M. Adbel-Hamid	Visiting Fellow	STB/TRTP NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS; Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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 (200 words or less - underline keywords)

The primary long-term goal of this work has been to correlate structure-activity relationships for halogenated hydrocarbons and determine how the degree and position of halogenation effects the absorption disposition and bioaccumulation of these compounds. This work has established that whereas polar halogenated hydrocarbons may be excreted prior to metabolism simple aryl halides are not excreted prior to metabolism to polar compounds and the rate of metabolism is limited by the availability of adjacent unsubstituted carbon atoms which are thought to facilitate metabolism via arene oxide intermediates. This work has also established that halogenated aromatics are readily absorbed from the gastro-intestinal tract, that those compounds which are not polar or are not readily metabolized will persist in the tissues, that chronic exposure to persistent halogenated aromatics will result in bioaccumulation to toxic levels and that the ability to metabolize halogenated aromatics varies widely with species. The studies of metabolism have demonstrated that acute as well as chronic toxicity may be related to the exposed animals ability to metabolize and excrete the toxic compound.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: This work has utilized ^{14}C-labeled compounds to quantitate absorption, distribution, accumulation metabolism and excretion of a series of nine polychlorinated biphenyls (PCBs), a polybrominated biphenyl (PBB), two insecticides and a tetrachlorodibenzofuran (TCDF). PCBs have been studied in mice, rats and monkeys; TCDF has been studied in rats, guinea pigs and monkeys; and the PBB and insecticides have been studied in rats. Studies of xenobiotic disposition have been conducted under conditions of normal feeding and starvation as well as acute versus multiple exposure. Analyses were facilitated by the use of a biological material's oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Studies of a uniquely toxic PCB, 3,4,3',4'-tetrachlorobiphenyl (TCB), in male and female rats and female monkeys have shown this PCB to be metabolized and excreted at a rate which is intermediate between that of rapidly metabolized and persistent PCBs which have been the subjects of past studies. No appreciable sex related difference was observed in the disposition of TCB in rats. A species related difference was observed for the disposition of TCB in a comparison of rats and monkeys. The biological half-life of TCB in the monkey was approximately fivefold that observed for the rat. The major difference observed for TCB vs. other less toxic PCBs is that a metabolite of TCB persists in the blood longer than any other PCB or PCB metabolite studied to date.

2) A study of the metabolism and disposition of chlorendic acid demonstrated that this compound was readily absorbed from the gastrointestinal tract, rapidly distributed to all tissues, most concentrated in liver and rapidly excreted, primarily in feces, with a half-life of less than one day. Chlorendic acid is structurally similar and equally chlorinated as the persistent insecticides aldrin and dieldrin, but chlorendic acid is much more polar than the insecticides due to the presence of two carboxilic acid groups. Therefore, the results of this study indicate that lipid solubility and resistance to metabolism to polar compounds contributes to persistence and bioacccumulation more than does simple halogenation.

3) The findings described here and in previous years have provided the basis for the following conclusions for simple halogenated aromatics: a) their lipid solubility facilitates absorption, b) their half-lives are determined by the polarity rate at which they are metabolized to polar compounds, c) their metabolism is determined by the position rather than the degree of halogenation, d) the major sites of accumulation are the liver, skin and adipose tissue and the relative importance of the liver and adipose tissues as storage sites is determined by the polarity of the compound in question.

PROPOSED COURSE: Studies done to date have firmly established those factors which determine the disposition of simple halogenated aromatics. Future studies will be designed to expand this base of knowl ge by determining how other types of substitutions, in addition to halogens, effect the metabolism and disposition of organic compounds. In addition, the effort to elucidate the biochemical mechanism(s) by which halogenated hydrocarbons exert their action will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Halogenated xenobiotics are the most toxic and persistent of the chemicals contaminating our environment. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and numerous industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man and may lead to more specific modes of treatment including better ways to accelerate removal of such compounds from the body.

PUBLICATIONS

Kato, S., McKinney, J. D. and Matthews, H. B.: Metabolism of symmetrical hexachlorobiphenyl isomers in the rat. Toxicol. Appl. Pharmacol. 53: 389-398, 1980.

Matthews, H. B. and Tuey, D. B.: The effect of chlorine position on the distribution and excretion of four hexachlorobiphenyl isomers. Toxicol. Appl. Pharmacol. 53: 377-388, 1980.

Tuey, D. B. and Matthews, H. B.: Use of a physiological compartmental model for the rat to describe the pharmacokinetics of several chlorinated biphenyls in the mouse. Drug Metab. Dispos. 8: 397-403, 1980.

Tuey, D. B. and Matthews, H. B.: Distribution and excretion of 2,2',4,4',5,5'-hexabromobiphenyl in rats and man: Pharmacokinetic model predictions. Toxicol. Appl. Pharmacol. 53: 420-431, 1980.

Matthews, H. B.: Chapter 8: Elimination of toxicants and their metabolites. In Hodgson, E. and Guthrie, F. E. (Eds.): Introduction to Biochemical Toxicology. Elsevier, Amsterdam, 1980, pp. 162-179.

Abdel-Hamid, F. M., Moore, J. A. and Matthews, H. B.: Comparative study of 3,4,3',4'-tetrachlorobiphenyl in male and female rats and female monkeys. J. Toxicol. Environ. Hlth. 7: 181-191, 1981.

Matthews, H. B.: Metabolism of Aryl Halides. In Jakoby, W. B., Bend, J. R. and Caldwell, S. (Eds.): Metabolic Basis of Detoxication. Academic Press, Inc., New York. In Press.

Matthews, H. B.: Disposition of Persistent Halogenated Hydrocarbons in Higher Animals. In Khan, M. A. Q. (Ed.): Symposium on Health and Ecological Effects of Chlorinated Hydrocarbons. Pergamon Press Inc., New York. In Press.

University of Oregon Health Sciences Center
School of Medicine
Department of Pharmacology
NO1-ES-7-2126

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: Robert K. Lynn, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, EBB

DATE CONTRACT INITIATED: September 30, 1977

CURRENT ANNUAL LEVEL: $157,790

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or scientists in the intramural program at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner, and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS:

1) A series of bisazobiphenyl dyes derived from benzidine, 3-3'-dimethylbenzidine or 3,3'-dimethoxybenzidine were studied in the dog and rat. Dogs treated acutely (100 mg/kg) with benzidine derived dyes excreted substantial quantities of benzidine (166-1675 μg) in urine (0-48 hr). Benzidine present in dog urine following dye administration exceeded by at least nine-fold the benzidine present as impurity in the administered dyes and was comparable to that excreted in urine when pure benzidine was fed (100 mg/kg). Rats chronically dosed (100 mg/kg/day) with benzidine-based dyes excreted N-acetylbenzidine (3-54 μg/day) and traces of benzidine in urine. Bisazobiphenyl dyes derived from 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine were metabolized to 3,3'-dimethyl and 3,3'-dimethoxybenzidine, respectively, in both the dog and rat. Results from this study indicate that the metabolic conversion of bisazobiphenyl dyes, derived from benzidine and 3,3'-dimethyl- and 3,3'-dimethoxybenzidine, to a known or suspect carcinogens is a general phenomenon and therefore with few exceptions should be anticipated for each member of this class of chemicals.

2) Detailed metabolism, distribution and excretion studies with ^{14}C-benzidine have been completed in the rat. Excretion studies demonstrated that a major portion (up to 75%) of the orally or intravenously administered radiolabel had been excreted in the feces three days after dosing. Distribution studies in all major tissues revealed substantially higher concentrations of radiolabel in the liver than in other tissues at all time (0.5, 1, 2, 4, 8, 24 and 72 hr) studied. Urinary and biliary metabolites of benzidine have been identified by gas-chromatography mass spectrometry. The major radiolabeled components in urine and bile are polar materials, several of which are glucuronide conjugates of benzidine, N-acetylbenzidine, N,N'-diacetylbenzidine and N-acetylhydroxybenzidine. In vitro studies with the isolated perfused rat liver have shown that benzidine is rapidly acetylated by the liver.

3) Metabolism, distribution and excretion studies of 3,3'-dimethoxybenzidine are underway in the rat. As was observed for benzidine, the dimethoxy analogue was excreted largely in the feces following oral dosing. In animals with exteriorized bile flow approximately 70% of the intravenously administered radiolabel was excreted in bile. More than 20 radiolabeled components were separated in urine. In contrast to the prominence of N-acetylation in the metabolic fate of benzidine, the dimethoxy analogue was acetylated only to a minor extent at these doses. Glucuronide conjugates appear to account for a major portion of the dose in bile. Administration of ^{35}S-sulfate prior to dosing with dimethoxybenzidine resulted in the excretion of one ^{35}S labeled dimethoxybenzidine metabolite.

4) Direct Blue 6, a bis-azo-biphenyl dye, has been synthesized in this laboratory with ^{14}C label in the benzidine nucleus. The radiochemical purity of the product is greater than 95% as determined by HPLC.

PROPOSED COURSE: 1) An additional radiolabeled dye, Direct Blue 14, will be synthesized.

2) In depth studies of absorption, distribution, metabolism and excretion of radiolabeled Direct Blue 6, Direct Blue 14, 3,3'-dimethyl- and 3,3'-dichlorobenzidine will be done in the rat. Data from these studies will be used to calculate the kinetic parameters for the disposition of these compounds, and an effort will be made to extrapolate this data to the human condition in order to access the hazard posed by human exposure to dyes synthesized from these carcinogenic precursors.

3) Additional compounds or classes of compounds will be studied in support of the NTP and intramural research of the NIEHS as time, resources and needs dictate.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies of such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data which will complement studies of toxic xenobiotics under the NTP or in the NIEHS intramural program.

PUBLICATIONS

Lynn, R. K., Donielson, D. W., Ilias, A. M., Kennish, J. M., Wong, K. and Matthews, H. B.: Metabolism of bisazobiphenyl dyes derived from benzidine, 3,3'-dimethylbenzidine or 3,3'-dimethoxybenzidine to carcinogenic aromatic amines in the dog and rat. Toxicol. Appl. Pharmacol. 56: 248-258, 1980.

Lynn, R. K., Wong, K., Dickinson, R. G., Gerber, N. and Kennish, J. M.: Diester metabolites of the flame retardant chemicals, tris (1,3-dichloro-2-propyl) phosphate and tris (2,3-dibromopropyl) phosphate in the rat: Identification and quantification. Res. Comm. Chem. Pathol. Pharmacol. 28: 351-360, 1980.

Arizona Board of Regents
University of Arizona
Tucson, Arizona 85724
NO1-ES-8-2130

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, EBB

DATE CONTRACT INITIATED: September 15, 1978

CURRENT ANNUAL LEVEL: $143,436

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 similiarly, 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) A study of the metabolism and disposition of acrylamide demonstrated that following oral or iv administration acrylanide is rapidly distributed to all tissues. Clearance of acrylamide derived radioactivity from most tissues follows a biphasic, first-order exponential decay, with an initial half-life of approximately 5 hours and a much longer terminal half-life of approximately 8 days. The terminal half-life of acrylamide in spinal cord was exceptionally long, approximately 24 days, and may be related to the neurotoxic effects of this compound. The major route for acrylamide excretion was urine and most of the material excreted was in the form of various metabolites. The major metabolite excreted in urine was an n-acetyl-cysteine conjugate.

2) Disposition studies of chlorpheniramine maleate indicate that this antihistaminic is readily absorbed, rapidly metabolized and excreted primarily in the form of metabolites. Following absorption chlorpheniramine is distributed to all tissues with peak concentrations in liver, and the largest percent of the total dose being in muscle. Excretion was primarily in urine and relatively rapid, better than 80% of the dose was excreted within two days. These results indicate that if the human is able to clear chlorpheniramine as rapidly as the rat, therapeutic doses should pose little threat of bioaccumulation.

3) Parachloroaniline disposition was studied in the rat. There was no dose related effect on metabolism and disposition in the dose range studied (0.3 to 30 mg/kg). At all doses parachloroaniline was readily absorbed, rapidly metabolized and excreted primarily in urine. Parachloroaniline was most persistent in blood due to its binding to hemoglobin in red blood cells, at seven days post exposure most of the parachloroaniline retained in the body was bound to red blood cells.

4) Comparative studies of in vitro versus in vivo metabolism of three polychlorinated biphenyls (PCB's) have been conducted in three species and enzyme preparations from each species. It has been established that both in vivo and in vitro metabolism of PCB's is determined by the degree and position of chlorination. An effort is being made to accurately predict in vivo metabolism from results obtained in vitro with hepatic microsomal preparations or isolated hepatocytes. The goal of this work is to refine techniques which will permit an in vitro to in vivo extrapolation for laboratory animals and therefore facilitate the extrapolation of laboratory data to man.

PROPOSED COURSE

1) The work on in vitro versus in vitro versus in vitro metabolism and extrapolation will be continued.

2) The metabolism of 1,2,3-trichloropropane, p-nitrotoluene and o-nitroanisole will be studied in the rat.

3) A comparative study of the metabolism and disposition of triortho-, trimeta- and triparacresylphosphate will be done in the rat and at least one species which is sensitive to the neurotoxicity of triorthocresyl phosphate.

4) 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

Miller, M. J., Carter, D. E. and Sipes, I. G.: Pharmacokinetics of arylamide in Fisher 344 rats. Toxicol. Appl. Pharmacol. In press.

MOUNT SINAI SCHOOL OF MEDICINE - New York, NY 10029
(NIH-NO1-ES-9-0004)

TITLE: Investigation of the Immunobiological and Toxicological Effects of PBB in Michigan Farmers and Chemical Workers

CONTRACTOR'S PROJECT DIRECTOR: J.G. Bekesi, Ph.D. and I. Selikoff, M.D.

PROJECT OFFICER (NIEHS): J.H. Dean, Ph.D., Research Immunologist/ Microbiologist, Environmental Biology Branch

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL: $500,000

PROJECT DESCRIPTION

OBJECTIVES: Altered immune function has been reported following exposure to PBB in humans and rodents. Altered immune function is but one of several symptoms and conditions that have been reported in PBB exposed persons. Thus far, investigation of immunological dysfunction and symptoms, in conjunction with various measures of PBB exposure have not shown a clearly defined dose/ response relationship. This may be because: 1) no such relationship exists; 2) current methods of estimating exposure based on body burden are inadequate for this purpose; 3) such a relationship will only emerge after considerable time has passed, or 4) only persons susceptible for other reasons show immunotoxicity.

The specific objectives of this study are as follows: 1) To verify the existence of previously reported immune dysfunction in an expanded population of Michigan farmers and chemical workers; 2) To relate any disturbance of immunity to detailed measures of PBB exposure, either historical or biochemical; 3) To investigate whether other reported symptoms, signs or conditions occur more frequently in those persons with immune dysfunction than those without; and, 4) To characterize and define the underlying mechanism of the immune dysfunction observed to date.

METHODS EMPLOYED: It is proposed that starting in the spring of 1980 a portion of the original 250-300 farm personnel examined in the 1976 Mt. Sinai survey or a similar group constitute a Michigan population group exposed to PBB through ingestion of food products be studied. Approximately 50-75 Wisconsin residents will also be evaluated for control (not exposed to PBB) purposes. In addition, a population of up to 90 Michigan chemical workers will be evaluated to compare individuals who were directly exposed to PBB and those who realized their PBB exposure principally through ingestion of food products.

All individuals enrolled in this study will receive a standardized health evaluation with specific focus on parameters allegedly associated with PBB exposure. Specific focus parameters will comprise the following: immunologic evaluation; liver function, to include standard clinical chemistry enzymes; neurological and/or neuropsychiatric evaluation; to include specific attention to the relatively unique secondary hypersomnia that has been reported; qualitative and quantitative evaluation of porphyrins in urine; dermatologic examination; and to establish serum and fat organohalide body burdens (PBB, PCB, DDT,

DDE, etc.) to correlate health status, including immune alterations with any or all chemicals that constitute the organohalogen body burden. Also, the contractor will conduct experiments on the qualitative and quantitative compartmentalization of PBB in specific subsets of lymphocytes and attempt to correlate these with immune alterations. Finally, an indepth characterization of the original population is proposed. The contractor will extensively evaluate people evidencing altered lymphocyte immune function with specific focus on a variety of factors such as, responsiveness of null cells to thymosin, extensive characterization of various T-lymphocyte subsets from a surface marker and functional standpoint, characterization of macrophage function, and investigation of the role of humoral serum factors.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year immunological profiles and clinical evaluations were performed on 336 PBB-exposed Michigan dairy farmers and 29 Michigan chemical workers, along with a low level exposure group from the general population (117) and two negative control groups, one from the Michigan general population (48) and the other Wisconsin dairy farmers (41). The immunological data were subjected to peer review for technical soundness and possible clinical significance by two outside renown clinical immunologists and the Project Officer during February 1981. The peer reviewers were satisfied with the technical quality of the data as well as the specimen collection, transport and double blind coding. The most significant in vivo observation was an enhanced delayed cutaneous hypersensitivity response to recall antigens (streptococcal and mumps antigens) in up to 50% of the exposed group. In vitro analysis of serum and lymphocytes revealed elevated immunoglobulin levels (IgG, IgM and IgA) in up to 30% of the exposed population, depressed lymphoproliferative responses to T-cell mitogens and allogeneic leukocytes, decreased number of thymus-dependent lymphocyte with an accompanying increase in cells lacking neither T- or B-cell markers (so called "null cells") in a moderate proportion of PBB exposed individuals.

The combined clinical and laboratory immune dysfunctions reported are preliminarily consistent with a defect in the regulation of immunoglobulin synthesis which could possibly result from the loss of a regulatory thymus dependent lymphocyte. One problem in interpretating these findings is the failure to have a statistically segregation of the various clinical findings by individual. This aspect has been recommended to the contractor for completion during the next contract year.

It is suggested that a few individuals possessing the greatest level of immune dysfunction should be evaluated in greater detail at the Mt. Sinai Medical Center. Although it is premature to consider possible clinical syndromes that might develop on a short or long term basis it appears that some form of immune dysfunction is associated with PBB exposure in these individuals and has persisted in a few since their previous evaluation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The complex relationship between PBB exposure, current PBB body burden and altered immune function in man remains yet undefined. The health implications of the altered skit test reactivity, elevated immunoglobulin levels, altered lymphocyte surface marker expression and the observed T-lymphocyte dysfunction may have important long-term health consequences on immune surveillance against infectious agents, neoplastically transformed cells and autoimmune diseases.

PUBLICATIONS

Bekesi, J.G., Anderson, H., Roboz, J.P., and Selikoff, I.J.: Investigation of the immunobiological effects of PBB in Michigan farmers. In Dean, J.H., and Padarathsingh, M.L. (Eds.): Biological Relevance of Immunocompetence. New York, Van Norstrand Reinhold, 1981.

Anderson, H.A., Wolff, M.S., Lilis, R., Holstein, E.C., Valciukas, J.A., Anderson, K.E., Petrocci, M., Sarkozi, L., and Selikoff, I.J.: Symptoms and clinical abnormalities following ingestion of polybrominated-biphenyl-contaminated food products. Ann. New York Acad. Sci. 320: 684-702, 1979.

NORTHROP SERVICES, INC. - Research Triangle Park, NC 27709
(NIEHS NO1-ES-79-0009)

TITLE: Animal Research on the Inhalation Toxicology of Environmental Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Bernard Adkins, Ph.D.

PROJECT OFFICER (NIEHS): E.W. Van Stee, D.V.M., Ph.D.

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL (5 years): $2,663,653.00

PROJECT DESCRIPTION

OBJECTIVES: Conduct research in the inhalation toxicology of environmental chemicals using dynamic flow-through inhalation chambers designed for use with small laboratory animals. Exposures are conducted intermittently since the inhalation facility is not equipped for 24-hour inhalation exposures. Generate, monitor, characterize and control the generation of solid aerosols of asbestos and related natural and man-made fibers in 1-4 inhalation chambers as specified to support the research program of the Laboratory of Pulmonary Function and Toxicology. Design and place into operation a computer-assisted augmentation of the existing gas inhalation facility based on concepts and specifications provided by the Government. Conduct a two-year oncogenesis study in which groups of rats and hamsters receive NO_2 by inhalation and 2,6-dimethylmorpholine (DMM) in the drinking water, air plus DMM, NO_2 and plain drinking water, or air plus plain drinking water. Conduct a 6 month oncogenesis study in which Strain A/J mice are exposed to (or treated with) all combinations of 4 concentrations of NO_2 (0, 1, 5, 10 ppm) in air, 4 concentrations of morpholine (0, 0.01, 0.05, 0.10%) in the drinking water, and 2 levels of alpha-tocopherol (0, 200 mg/kg) in an AIN-76 diet. Install a scrubbing system capable of reducing the concentration of vinyl chloride from the effluent from one chamber from 5000 ppm to 5 ppm. Conduct a 30 day exposure of mice to 5000 ppm of vinyl chloride.

MAJOR FINDINGS AND PROPOSED COURSE: Site preparation by the Government is in progress and the development of the computer system is continuing as fast as the renovation proceeds. Completion of the Government renovation is expected to extend well into 1982. Computer-assisted operation of the facility should be well-along by 1982. Detailed protocols for the NO_2-DMM and Strain A/J mouse studies have been written and reviewed. The NO_2-DMM exposures will be completed during 1983. The Strain A/J mouse exposures will be ended about August 15, 1981. The goals of the Strain A/J mouse study are 2-fold: (1) establish the dose-response relationship of NO_2 and morpholine to the formation of pulmonary adenomas, and (2) determine if the administration of alpha-tocopherol modifies pulmonary adenomatosis that results from concurrent exposure to NO_2 and morpholine. If the NO_2-morpholine dose-response relationship is established in the preceding experiment, other groups of animals will be exposed to an optimal combination of NO_2 and morpholine and also treated with various potential modifiers of chemical oncogenesis, e.g., alpha-tocopherol (repeat), retinoids, ascorbate, BHT, and magnesium for the purpose of identifying possible means for protection against the potential oncogenic effects of concurrent exposure to NO_2 and nitrosatable amines.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The implementation of computer-assisted inhalation facility operation represents an attempt to bring inhalation technology closer to present-day laboratory computer technology. Computer-assisted operation greatly enhances the accuracy and flexibility of the operation of the inhalation facility as well as the documentation of that operation, and greatly reduces manpower requirements, thus reducing the labor costs of facility operation. The potential in vivo interaction of NO_2 with heterocyclic amines represents a novel concept in environmental carcinogenesis. That detectable quantities of potentially carcinogenic nitrosamines can be formed in the bodies of laboratory animals that are given morpholine by gavage and exposed to NO_2 by inhalation has been reported. The biological significance of the phenomenon has been suggested by preliminary studies conducted at NIEHS. The results of these studies suggest the existence of a specific chemical link between exposure to NO_2 and tumor formation in mice (and possibly rats). Further, these studies pave the way for efforts to find ways to interfere with elements of the in vivo process of oncogenesis with the long-term goal of identifying possible protective measures that might be taken to reduce the effects of this potential environmental hazard.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE - Chicago, Illinois 60616
(NO1-ES-1500)

TITLE: Chemical Induced Immunotoxicity Studies

CONTRACTOR'S PROJECT DIRECTOR: Dr. James Fenters

PROJECT OFFICER (NIEHS): J.H. Dean, Ph.D., Research Immunologist/
Microbiologist, Environmental Biology Branch

DATE CONTRACT INITIATED: February 1, 1981

CURRENT LEVEL: $285,328

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to develop and validate with another contract laboratory an immunological and host resistance panel for defining immunotoxicity following chemical exposure.

METHODS EMPLOYED: To achieve these objectives, the contractor will evaluate methods for detecting altered host resistance to bacteria, viruses, parasites and transplantable tumor cells following chemical exposure and define a reproducible susceptibility test panel. The contractor will perform and improve immunological assays. In parallel with host resistance assays, chronically exposed B6C3F1 mice will be evaluated to define immunological alterations and correlated with host resistance changes. Three chemicals will be evaluated in parallel with a second contractor using different host resistance and similar immune function assays during the methods development and selection phase of the contract. During the correlation phase in which the selected panel will be evaluated, 5 chemicals suspected of immunotoxicity will be evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: Since this contract was only awarded on Feburary 1, 1981, sufficient time has not elapsed to evaluate its progress. To date, facilities alterations have been completed and research has begun. During the coming year the host resistance methods development will proceed and the immune function assays validated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract is of extreme importance and relevant to environmental health medicine since it should provide methods selection development and data base necessary for devising better human clinical field trials where exposure to environmental chemicals is suspected of altering host resistance or immunological function. These data may also help substantiate the increased sensitivity of the immune system for defining chemical toxicity and to define the magnitude of immunological change necessary to alter host resistance.

MEDICAL COLLEGE OF VIRGINIA - Richmond, Virginia 23298
(NO1-ES1-5001)

TITLE: Chemical Induced Immunotoxicity Studies

CONTRACTOR'S PROJECT DIRECTOR: Dr. Page Morahan

PROJECT OFFICER (NIEHS): J.H. Dean, Ph.D., Research Immunologist/ Microbiologist, Environmental Biology Branch

DATE CONTRACT INITIATED: February 1, 1981

CURRENT LEVEL: $242,928

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to develop and validate with another contract laboratory an immunological and host resistance panel for defining immunotoxicity following chemical exposure.

METHODS EMPLOYED: To achieve these objectives, the contractor will evaluate methods for detecting altered host resistance to bacteria, viruses, parasites and transplantable tumor cells following chemical exposure and define a reproducible susceptibility test panel. The contractor will perform and improve immunological assays. In parallel with host resistance assays, chronically exposed B6C3F1 mice will be evaluated to define immunological alterations and correlated with host resistance changes. Three chemicals will be evaluated in parallel with a second contractor using different host resistance and similar immune function assays during the methods development and selection phase of the contract. During the correlation phase in which the selected panel will be evaluated, 5 chemicals suspected of immunotoxicity will be evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: Since this contract was only awarded on Feburary 1, 1981, sufficient time has not elapsed to evaluate its progress. To date, facilities alterations have been completed and research has begun. During the coming year the host resistance methods development will proceed and the immune function assays validated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract is of extreme importance and relevant to environmental health medicine since it should provide methods selection development and data base necessary for devising better human clinical field trials where exposure to environmental chemicals is suspected of altering host resistance or immunological function. These data may also help substantiate the increased sensitivity of the immune system for defining chemical toxicity and to define the magnitude of immunological change necessary to alter host resistance.

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 the 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 and private institutions 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/or analysis issues arising in laboratory experimentation, with special emphasis on toxicological screening assays, and provide a comprehensive consulting service for the epidemiological component of the Biometry and Risk Assessment Program, and the National Toxicology and Intramural Research Programs. The Epidemiology Branch initiates field studies of human disease, particularly chronic diseases, due 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 provides computing support to laboratory research activities in various branches; and provides systems analysis and project management support to both Institute and NTP system development projects.

STATISTICS AND BIOMATHEMATICS BRANCH

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

Research projects in two separate areas of mathematical biology are currently in progress. Both of these programs are collaborative efforts with other research groups in the Intramural Research Program. The objective of the joint research with the Laboratory of Animal Genetics is to develop mathematical models for phenomena in population genetics. An ongoing project has been to develop models to estimate nucleotide substitution rates from restriction enzyme map data as well as nucleotide sequence data. Additional work is underway to determine how the estimates are effected by insensitivities of some of the laboratory techniques. Work has also commenced on constructing models for the evolution of transposable elements in DNA. A simulation study is underway to study this phenomena; and in the case of infinite recombination, some analytic progress has been made.

Efforts are also being made to improve and modify existing models that predict the number of mutant colonies in growing bacterial populations. These models are very useful in analyzing data obtained from short term in vitro tests for chemical mutagenicity. While much work has already been done on the Ames test, several mammalian cell test systems are under study.

The major emphasis in risk assessment research is on the development and analysis of statistical procedures for assessing human cancer risk from laboratory animal based data. Various research projects focused on different aspects of this problem are being conducted within the Statistics and Biomathematics Branch.

The Gamma multi-hit model for low dose risk estimation was evaluated using data generated in a variety of experimental settings. This assessment raised serious concerns about both the "safe dose" level estimates and the associated confidence limits that can be obtained with the Gamma multi-hit approach.

The Armitage-Doll multistage model and various competitor low-dose extrapolation techniques are also being evaluated using the massive data base produced by NCTR's ED_{01} study of 2-AAF. In addition to determining the goodness-of-fit of the procedures under consideration, attempts are being made to assess the adequacy of the multistage model for predicting lifetime cancer risk when exposure is actually terminated well before the occurrence of death.

Work on the incorporation of pharmacokinetics into low-dose risk extrapolation is being continued with particular attention on the estimation of the error that can be introduced into the modeling process by not taking pharmacokinetic considerations into account.

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. In the data analysis area the Branch provides support which includes tabulation of summary statistics, curve fitting, significance testing, and interpretation of test results. These efforts are closely co-ordinated with the computing work group.

During the past year, considerable attention has been devoted to statistical problems that have arisen as a result of consulting activities related to the National Toxicology Program's (NTP's) *in vitro* and *in vivo* testing programs. In the analysis of tumor incidence data from the NTP bioassays, particular emphasis is now given to procedures that adjust for survival differences. Statistical methodology is being developed that will take extra-binomial variability in historical control tumor incidence into account, and will allow the incorporation of this information into a formal significance test. In the area of *in vitro* testing, research efforts are being focused on the adequacy of a family of models to fit Ames test data. Statistical methods to detect non-Poisson sampling behavior in count data derived from genetic toxicological experiments are also being investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40002-11 SBB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph K. Haseman	Mathematical Statistician	SBB	NIEHS
Other:	Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
	Beth C. Gladen	Statistician	SBB	NIEHS
	Joo O. Koo	Visiting Fellow	SBB	NIEHS
	Clovis A. Peres	Visiting Associate	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:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was undertaken to develop new statistical methodology to deal with a variety of problems related to the Branch's consulting activities. Specific areas in which statistical research is being conducted include analysis of binary responses, mathematical modeling for dominant lethality data, and randomized complete block designs.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques ranging from Monte Carlo simulation procedures to analytical test development and mathematical modeling have been employed to address various statistical methodology problems arising from the Branch's intramural consulting activities.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Various procedures for comparing two groups producing beta-binomial responses were evaluated by computer simulation. The tests included t-tests on various transforms, a Mann-Whitney test, randomization tests, a Jackknife-based test, and a weighted least squares procedure. It was found that in most instances, power differences among the tests were minor. (2) Evaluation of the incomplete beta function ratio arising in the cumulative distribution function of the binomial, negative binomial, student t and F distributions has been investigated. For these four probability density functions, the ratios have parameter values that are integer multiples of 1/2, and in this case there is a closed-form solution. (3) In the randomized complete block design, the block system causes, for many applications, strong correlation between responses from experimental units in the same block. In these instances, the assumptions underlying the usual statistical analysis based on this design are violated. It was shown that the usual F test for testing treatment effects can be used even if there is a constant covariance between responses from experimental units in the same block. Approximate solutions for testing treatment effects were also derived for the case in which the variances associated with each treatment are not the same.

Statistical methodological research will continue to form an important part of the overall support effort provided to the Intramural Research Program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of improved statistical methodology is essential if the Branch is to provide effective statistical consulting to the Intramural Research Program.

PUBLICATIONS

Poon, A.H.: A Monte Carlo study of the power of some k-sample tests for ordered binomial alternatives. J. Statist. Comput. Simul. 11: 251-259, 1980.

Peres, C.A.: Testing the effect of blocking in a randomized complete block design (RCBD). Commun. Statist.-Theor. Meth., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40004-04 SBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Statistical Methods in Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Beth C. Gladen	Statistician	SBB	NIEHS
Other:	Allen J. Wilcox	Medical Officer (Research)	EB	NIEHS
	Robert I. Jennrich	IPA	SBB	NIEHS
	David G. Hoel	Chief	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:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct reseach 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.

PHS-6040
(Rev. 2-81)

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) In the study of spontaneous abortions, age, gavidity and prior abortions have been proposed as explanatory factors for current abortions. Models have been formulated that attempt to separate the roles of these factors. Computer simulations were performed to determine the consequences of various assumptions and to see how closely various models can reproduce the real experience of women. (2) A study of life table methods for epidemiological data involving deaths from a relatively rare disease was undertaken. Interest centered on the reliability of standard statistical tests in a setting where, although total sample sizes are large, the number of deaths due to the disease of interest may be small. It was shown that standard asymptotic tests including the log-rank and generalized Wilcoxon homogeneity and trend tests can overestimate statistical significance. To obtain a more accurate assessment of significance two alternative methods were considered. The first involved the use of permutation, rather than asymptotic, tests. While the permutation tests are exact when the censoring intensities are equal, this is seldom the case in practice. Indeed, the permutation tests can be fairly sensitive to inequalities in censoring intensity. As a consequence they must, in practice, be viewed as approximate tests. In light of this a new approximate test was proposed and studied which is less sensitive to unequal censoring than the permutation test while being less sensitive to small sample failures than the asymptotic tests. The new test, called a simulation test, is based on simulating the conditional distribution of groups in which a tumor is found given the numbers at risk in the various dose groups at the time of the tumor. It is simpler to implement than the permutation tests and seemed to give about the same results as the permutation test for the data set considered in this investigation.

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.

PUBLICATION

Takagi, H.: An algorithm for causal modeling in epidemiological studies. Behaviormetrika 8: 41-55, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40005-04 SBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Statistical Analysis of Mutagenesis Testing Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Other:	Norman Kaplan	Research Mathematician	SBB	NIEHS
	Bruce Collings	Mathematical Statistician	SBB	NIEHS
	Ken Risko	Mathematical Statistician	SBB	NIEHS
	Errol Zeiger	Head, EMTDP	LMG	NIEHS

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics

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 (200 words or less - underline keywords)

The long-term objective of this ongoing project remains the development of appropriate statistical techniques for the analysis of data arising from assays under study in the Environmental Mutagen Test Development Program (EMTDP). Results are also applicable to other large mutagenicity studies, such as the European Collaborative Study and the International Program for the Evaluation of Short-Term Tests for Carcinogenicity. The major focus to date has been on microbial test systems, although preliminary work has now commenced on Drosophila and two mammalian cell systems, CHO and L5178Y. Statistical procedures for the design and analysis of short-term tests proposed or currently employed by other researchers in mutagenicity continue to receive scrutiny, and new and improved procedures continue to be devised.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Test for mutagenicity remains under study, especially regarding the influence of protocol parameters upon the observed response. A mixture of mechanistic and empirical modelling, together with statistical data analytic procedures, has constituted the main methodological approach toward the microbial systems, and also is the approach adopted toward the mammalian cell and Drosophila assays.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The set of models developed for Ames Test data provides a mutagenic index that summarizes and quantifies up to protocol uncertainties the results obtained from a single-strain Ames Test. This index lends itself to studies of response reproducibility, both within and across laboratories, and inter-strain agreement. (2) The analysis of an EMTDP database on over 200 chemicals has commenced to study both the adequacy of the set of models developed, and to investigate sources of variability in response, with an eye toward introducing quality control procedures.

SIGNIFICANCE TO 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., Kaplan, N., and Zeiger, E.: Statistical analysis of the Ames salmonella/microsome test. Proc. Natl. Acad. Sci. (USA) 78(6): 1981.

Collings, B.J., Margolin, B.H., and Oehlert, G.W.: Results for binomial data, with application to the design and analysis of fluctuation tests for mutagenicity. Biometrics, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41001-07 SBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Risk Assessment for Environmental Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Hoel	Chief	SBB	NIEHS
Other:	Kenneth G. Brown	IPA	SBB	NIEHS
	Joseph K. Haseman	Mathematical Statistician	SBB	NIEHS
	Michael D. Hogan	Mathematical Statistician	SBB	NIEHS
	Robert I. Jennrich	IPA	SBB	NIEHS
	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
	Marshall W. Anderson	Mathematician	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

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 (200 words or less - underline keywords)

The primary focus of this project is on the development of improved statistical techniques for estimating potential human cancer risk using data generated from animal laboratory experiments. Current research efforts are particularly concerned with an assessment of the Gamma multi-hit model and an evaluation of the performance of the multistage model in a megamouse experimental framework. The adaptation of methods for species-to-species extrapolation and the incorporation of pharmacokinetics in low-dose risk estimation also continue to be of interest.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Since this project involves the development of statistical procedures that are applicable to risk assessment issues, particularly in the area of carcinogenesis, a great deal of emphasis is given to modelling techniques. These techniques range from empirical data evaluation and curve fitting to the generation of mathematical models that attempt to approximate biological mechanisms.

MAJOR FINDINGS AND PROPOSED COURSE: It was found that the Gamma multi-hit model, which was recommended for use in risk assessment by the Scientific Committee of the Food Safety Council, can produce both dangerously high and unrealistically low "safe dose" estimates. Furthermore, neither the "standard" nor the "conservative" confidence limits associated with this model seem adequate for practical application.

The goodness-of-fit of the Armitage-Doll multistage model to the experimental data obtained from the NCTR ED_{01} study of 24,000 mice exposed to the known carcinogen 2-AAF is being evaluated. Parameter estimates derived from modelling of lifetime exposure data will be used to generate risk prediction equations for a subset of the study population for which dosing was discontinued several months prior to sacrifice. Finally, the performance of alternative low dose extrapolation models will be compared to the multistage model.

Research is continuing in the area of species-to-species extrapolation for both carcinogenic and non-carcinogenic endpoints, and the implications of incorporating pharmacokinetics into models which are used for estimating carcinogenic risk are being assessed. Attempts will be made to determine which are the most crucial pharmacokinetic parameters in cancer risk evaluation and to quantify the magnitude of the error that can be expected if pharmacokinetic considerations are inappropriately ignored.

Details of other risk assessment related research are presented in Project No. Z01 ES 45001-01 BB.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: As animal experimentation plays an ever-increasing role in the assessment of human cancer risk, the importance of improved statistical/mathematical procedures for realistically assessing this risk is obvious.

PUBLICATIONS

Anderson, M.W., Hoel, D.G., and Kaplan, N.L.: A general scheme for the incorporation of pharmacokinetics in low-dose risk estimation for chemical carcinogenesis: example--vinyl chloride. Toxicol. Appl. Pharmacol. 55: 154-161, 1980.

Haseman, J.K., Hoel, D.G., and Jennrich, R.I.: Some practical problems arising from use of the Gamma multi-hit model for risk estimation. J. Toxicol. Environ. Hlth., in press.

Hoel, D.G.: Extrapolation of laboratory data to human health effects. In Proceedings of the Brookhaven National Laboratory Symposium on Genetic Effects of Airborne Agents, Upton, New York, February 9-11, 1981, in press.

Hoel, D.G.: Extrapolation models of animal toxicity data to man. In Proceedings of the Conference on Environmental Risk Assessment: How New Regulations Will Affect the Utility Industry, New Orleans, Louisiana, December 10-11, 1980. Richland, Sigma Research, Inc., in press.

Hoel, D.G.: Statistical measures of risk. In Proceedings of the Symposium on Metabolism and Pharmacokinetics of Environmental Chemicals in Man, Sarasota, Florida, June 7-12, 1981. Drug Metabolism Reviews, in press.

Peres, C.A. and Koo, J.O.: A comparison of the two-component and quadratic models to assess survival irradiated stage-7 oocytes of drosophila melanogaster. Mut. Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-06 SBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Other:	Charles H. Langley	Research Geneticist	LAG	NIEHS
	Kenneth J. Risko	Mathematical Statistician	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:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is to develop mathematical models for phenomena encountered in population genetics. This work is being done in collaboration with scientists in the Laboratory of Animal Genetics. The current two areas of research are (1) continued investigation of models used to predict nucleotide substitution rates from restriction enzyme map data and DNA sequence data and (2) the development of simulation and analytic models for the study of the population genetics of transposable elements.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Collaborative research was conducted on problems in population genetics with scientists in the LAG. This research, which augments the population genetics studies performed by the LAG, was probabilistic in nature, emphasizing Markov chain theory and diffusion techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Some additional properties of the models developed to estimate nucleotide substitution rates have been examined. An algorithm has been developed which constructs an appropriate ancestral tree from DNA sequence data. The ramifications of low sensitivity gels has also been investigated. (2) The modeling of the evolution of transposable elements has been in two directions. Because of the difficulties of incorporating a nonlinear control mechanism, the process has been examined through a simulation. This work provided some indications as to how the process behaves. Analytically, the process has been modeled as a population-size dependent branching process. This model has enough salient features to make it interesting. One property of the model is that it is diffusable. Future work is to study the diffusion and attempt to incorporate other biological features into the model.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 45001-01 SBB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Experimental Design and Data Analysis Methodology for NTP Animal Bioassays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Hoel	Chief	SBB	NIEHS
	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Other:	Joseph K. Haseman	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Possible modifications of the basic experimental design of the National Toxicology Program's two-year carcinogenesis bioassay are being investigated. Changes being considered include additional dose levels, reallocation of animals among experimental and control groups, and the selection of dose levels other than the traditional MTD (maximum tolerated dose) and ½MTD. The purpose of these modifications would be to permit more precise risk estimates at low-dose levels without adversely affecting the power of the bioassay for detecting carcinogenic effects. Additional research efforts include the problem of incorporating historical control information into the statistical analysis of bioassay data.

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical methods including Monte Carlo simulation, mathematical modeling, non-linear optimization, asymptotic information theory and analytic test procedures were used to investigate possible modifications of the current cancer bioassay.

MAJOR FINDINGS AND PROPOSED COURSE: By comparing several bioassay designs with respect to the precision of low-dose risk estimation, it was possible to determine characteristics of designs which will give accurate extrapolations while maintaining power for carcinogenic detection when a multi-stage model of carcinogenesis is assumed. The results show that additional doses (beyond the standard three) generally do not increase the accuracy of the extrapolation. A Monte Carlo study of the model fitting procedure has yielded insight into the effect of fitting an incorrect model on low-dose risk estimation. The question of slope at dose zero is a very important issue which must be dealt with on the basis of biological considerations. Monte Carlo simulation studies are being continued to determine optimal designs for small sample bioassays.

Historical control tumor incidence rates frequently show considerable inter-laboratory variation. Even within a given laboratory, there is often more variability from study to study than would be expected under a simple binomial model. Statistical methodology is being developed that will take this extra-binomial variability into account and will allow the incorporation of historical control data into a formal significance test.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The goals of the National Toxicology Program's animal bioassay studies include the assessment of risk at low-dose levels as well as the detection of potential carcinogenic effects. If these objectives are to be achieved, it is essential that the basic design of the bioassay be re-examined, to determine if modifications are needed. Moreover, to increase the sensitivity of the bioassay, it is important to consider the utilization of historical control data.

PUBLICATIONS

Gaylor, D.W., and Hoel, D.G.: Statistical analysis of carcinogenesis data from chronic animal studies. In Sontag, J.M. (Ed.): Carcinogens in Industry and Environment. New York, Marcel Dekker, 1981, pp. 97-111.

Hoel, D.G.: Statistical issues in toxicology. In Proceedings of Environmetrics '81, Alexandria, Virginia, April 8-10, 1981. Society for Industrial and Applied Mathematics, in press.

EPIDEMIOLOGY BRANCH

EPIDEMIOLOGY BRANCH
Summary Statement

The research effort in epidemiology studies relationships between environmental exposures and human disease, including an active program developing epidemiologic, statistical, and laboratory methods that help make field studies more feasible and interpretable.

The Breast Milk and Formula Project is a prospective, birth cohort study of about 900 North Carolina children. Clinical data on growth, morbidity, and development is gathered on the children; levels of widespread contaminant chemicals, such as polychlorinated biphenyls and DDE (the stored metabolite of DDT) are measured in the mother at birth and in breast milk over time. Enrollment of children has been completed; a substanial number of the cohort is now two to three years old; most of the chemical analyses are complete; and data analysis has begun. The very high prevalence of breast milk contaminated with these chemicals (> 90%) has been confirmed; descriptive analyses of time trends have been published.

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 PCB's and other agents on the activity of placental enzymes), and outcomes at a cellular and molecular level (such as somatic cell mutations) that may indicate increased risk of an adverse health effect. The development and validation of assays will be integrated with their use in specific epidemiologic studies of environmental factors.

Program interest in the epidemiology of low birthweight and spontaneous abortion has continued. Methodologic issues in the description and analysis of fetal growth have been pursued; work is being done on mathematical modeling of spontaneous abortion and factors such as age at menarche and gravidity; these models will be compared with empirical data. A study of the validity of questionnaire responses concerning spontaneous abortion has been designed, and a study of laboratory methods for very early pregnancy detection and fetal loss is planned.

Demographic studies, using Vital Statistics data and indirect exposure assessments, have continued. This year, a study of liver cancer mortality by region, race, and hypothesized risk factors, such as aflatoxin exposure, was begun, as was a death certificate study of cancer mortality and occupation.

Two studies in metal toxicity have accomplished most of their data collection, and will proceed to data analysis. The first is a case-control study of selenium and other trace elements and skin cancer; the second is a study on the susceptibility of blood pigment (heme) formation to the effects of lead. An autopsy study of liver tissue of children dying from Reye syndrome, with analysis for aflatoxin, is about half complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43001-09 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Demographic Investigations of Potential Human Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dale P. Sandler	Statistician (Health)	EB	NIEHS
	Michael D. Hogan	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS (if any)

Statistics and Biomathematics Branch

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.30	0.20	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of demographic investigations. Current research activities involve a correlational analysis of suspected liver cancer risk factors and liver cancer mortality in the United States.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Potential environmental health hazards will be studied using available demographic and vital statistics data. Among other analyses time trends, cohort trends, and racial and geographic differences in liver cancer mortality will be explored.

MAJOR FINDINGS AND PROPOSED COURSE: Analysis of United States vital statistics data has shown that liver cancer death rates for non-white males are much greater than those for white males, although deaths from liver cancer are relatively rare in both groups. Similarly, non-white females have greater liver cancer mortality than do white females, and the rates for males of both racial groups are greater than those for females. The most striking finding was an increase over time in liver cancer mortality for non-white males. There were corresponding increases over time for other sex/race groups, however, these increases are slight and tend to occur in older age groups only.

Liver cancer death rates in 1969 and 1975 were higher in the southern portion of the United States than elsewhere. This is due largely to an excess for white males in the south. It is unusual and so far unexplained, that rates for non-white males are lower in the southern regions. Regional differences in liver cancer death rates do not correspond to regional death rates for cirrhosis or alcoholism or to regional differences in reported cases of hepatitis B. The non-white excess of liver cancer deaths does, however, correspond to an apparent excess of hepatitis B for non-whites and an excess of deaths due to cirrhosis and alcoholism. Additional explanations for racial and regional differences in liver cancer mortality are being sought using vital statistics data.

Future analyses will focus on liver cancer deaths in North Carolina only. Greater detail regarding hepatitis, alcohol consumption and other potential risk factors such as aflatoxin exposure is available on a state basis rather than for the United States as a whole.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preliminary analysis of the liver cancer death rates indicate that there may be some environmental factor which is unique to non-white males which makes a substantial contribution to liver cancer mortality in the United States. Liver cancer is fairly rare in the United States while it is relatively common elsewhere. Identifying the factor(s) responsible for the recent increase in mortality for non-white males may lead to an understanding of the causes of liver cancer and may help explain why liver cancer is rare in the United States.

PUBLICATIONS

Hogan, M.D. and Hoel, D.G.: Estimated cancer risk associated with occupational asbestos exposure. J. Risk Anal. 1(1): 67-76, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43002-05 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Breast Milk and Formula Project

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Walter J. Rogan	Medical Officer	EB	NIEHS
Other:	Beth C. Gladen	Statistician	SBB	NIEHS
	James D. McKinney	Supervisory Chemist	LEC	NIEHS
	Douglas B. Walters	Chemist	NTP	NIEHS
	Richard B. Everson	Epidemiologist	EB	NIEHS
	Thomas K. Wong	Staff Fellow	EB	NIEHS

COOPERATING UNITS (if any) Statistics and Biomathematics Branch;
Laboratory of Environmental Chemistry, NIEHS; Raltech, Inc., Madison, WI; Science Applications, LaJolla, CA; Wake Area Health Education Center, Raleigh, NC; Durham Women's Clinic, Durham, NC; East Carolina School of Medicine; Greenville, NC

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polychlorinated biphenyl (PCB) contamination of breast milk in the ppb range is well documented, but health effects on infants fed such milk are unstudied. This project involves: (1) establishment of a cohort of breast and formula fed infants; (2) development of methodology to obtain reliable and reproducible samples of human body fluids and tissue from mother and child; (3) development of reliable methods for analysis of PCB's and other related chemicals; (4) development and application of statistical procedures for the analysis of data generated from the study; and (5) evaluation of the children for specific outcomes thought to be related to organochlorine exposure including PCB's.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is a prospective, or follow-up study. Field personnel have been hired, trained in protocol administration, and work at selected hospitals. Subjects are enrolled, informed consent is obtained, and a questionnaire administered to each mother at approximately the time of delivery. Samples of milk, formula, colostrum, placenta, and maternal blood are collected.

These samples are subjected to gas chromatographic and neutron activation analysis for PCB's, DDE(1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane), total organic chlorine (TOCl) and bromine (TOBr), and total soluble organic chlorine (TSOCl) and bromine (TSOBr) in the ppb range. Some of the specimens of placental tissue will also be tested for levels of mixed function oxidase enzymatic activity.

The children are examined, and follow-up appointments made. Serial examinations including behavioral evaluations, are performed at birth, 6, 12, 18, 24 months, and then yearly over the first years of the child's life.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary data analysis is planned for summer 1981. Data collection in North Carolina will continue. Major progress to date has been enrollment of about 800 participants and the perfection of techniques required for collection of samples without possibility of contamination. Quality assurance (QA) procedures for control of chemical analyses and the development of methodology for reliable and reproducible ppb level determination of PCB, DDE, TOCl and TOBr have been completed for milk and blood. Similar QA work is continuing for formula and placenta (TSOBr and TSOCl only). Analysis of complete sets of mothers' samples has been initiated.

The remaining objectives are: (1) to establish the relationship between milk levels, maternal blood levels, cord blood levels, placental levels, and colostrum at birth, and examine the trends in milk concentration over time; (2) to investigate the relationship between PCB and TOCl levels in the neonate and a number of specific outcomes; (3) to follow breast-fed and non breast-fed infants over a period of years and look for differences in incidence of a number of specific outcomes; (4) to generate other hypotheses about toxic effects of chronic low dose PCB exposure in children; (5) to establish a cohort of children for follow-up studies; and (6) to determine whether there is an association between PCB or TOCl levels and placental mixed function oxidase activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health effects of these low dose environmental pollutants are not well studied in children, and this project should allow identification and quantification of those that occur short term in this group. The methodology for studying such phenomenon is also of interest, and the development of a field efficient method for study of low level pollutants, such as PCB's, in humans is important.

PUBLICATION

Rogan, W.J., Bagniewski, A.B., and Damstra, T.: Pollutants in breast milk. N. Engl. J. Med. 302: 1450-1453, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43003-03 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Pediatric Lead Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Walter J. Rogan	Medical Officer	EB	NIEHS
Other:	Beth C. Gladen	Statistician	SBB	NIEHS

COOPERATING UNITS (if any)
Statistics and Biomathematics Branch;
Medical University of South Carolina

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lead exposure and undue absorption occurs not only in inner city children exposed to paint but also in children around smelters and those whose parents work with lead. Although the encephalopathy and renal disease of acute lead intoxication are well described, other toxicities of lead as well as factors that alter susceptibility need further research. Investigation into genetic differences in susceptibility to the effects of lead on blood formation has been initiated.

PHS-6D40
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study of genetic variability in response to the toxicity of lead on blood formation was designed (and will be analyzed) in house, with data collection done on contract. The response variable is erythrocyte protoporphyrin (EP), the immediate precursor to heme in blood formation. Blood lead level is the exposure variable, and the amount and activity of amino levulenate dehydrase (ALA-D) the genetic marker. Children with known high or low responses of EP at a given lead level are selected from a prescreened population. They are revisited, and their blood lead, EP, and ALA-D levels are determined. About 200 hyper responders and 200 hypo responders will be tested. The hypothesis is that children who differ in their EP response will also differ in their ALA-D level.

MAJOR FINDINGS AND PROPOSED COURSE: We have identified the Medical University of South Carolina as a suitable data source, and have obtained records on the 6000 or so prescreened children in their program. We have constructed a stratified sample for revisit. They will find the children and do the blood work. Data gathering should be complete in the summer of 1981, and analysis early in 1982.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Lead is an ubiquitous pollutant, and children are most vulnerable to it. Lead absorption can be both prevented and treated. Thus, an understanding of its human toxicity is appropriately within the NIEHS mission. Current screening programs continue to find and treat children with undue lead absorption. The decision to treat is made on the basis of laboratory tests rather than clinical illness, and thus it is likely that some children are treated unnecessarily. Successful identification of children with greater susceptibility would allow more effective screening for children likely to show toxicity.

PUBLICATION

Rogan, W.J.: Some practical problems and solutions in lead poisoning prevention programs. In Chisolm, J.J., Jr. (Ed.): Management of Increased Lead Absorption Clinical, Social, and Environmental Aspects. Baltimore, Urgan and Schwarzenberg, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43004-03 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Environmental Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dale P. Sandler	Staff Fellow	EB	NIEHS
Other:	Richard B. Everson	Epidemiologist	EB	NIEHS
	Walter Rogan	Medical Officer	EB	NIEHS
	Allen J. Wilcox	Medical Officer (Research)	EB	NIEHS

COOPERATING UNITS (if any) School of Public Health and Memorial Hospital, University of N.C., Chapel Hill, School of Public Health, University of California, Berkeley, Wilson Dermatology Clinic, Wilson, N.C., Food and Drug Administration, Centers for Disease Control, Department of Epidemiology, School of Hygiene, Johns Hopkin Univ.

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves a number of studies of chronic disease or cancer epidemiology. It includes a preliminary investigation into the etiology of Reye's Syndrome; an investigation into the relationship of maternal smoking and subsequent cancer; and an investigation into the relationship of selenium and other trace elements to skin cancer.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: (1) Collaborative arrangements with two other agencies (CDC, FDA) have been established for the collection and analysis of tissue samples from children dying from Reye's Syndrome or other liver diseases. Aflatoxin B levels will be measured in order to determine if there is an association between Aflatoxin B exposure and pathologically confirmed Reye's Syndrome. (2) An epidemiologic study of the relationship of trace elements, particularly selenium, to the occurrence of skin cancer is being conducted in Wilson, North Carolina. Clinical data from patient examinations, historical data from questionnaires, and laboratory data from blood samples are being collected and analyzed to test the hypothesis of an inverse relationship between selenium and skin cancer. The interaction of other trace metals and vitamins will also be studied. (3) A case-control study of adult cancer risk and maternal smoking during pregnancy has been developed as part of a possible series of studies of potential human transplacental carcinogens. A patient population has been selected from the cancer registry at UNC Memorial Hospital. Controls will be identified by the cases themselves. Preliminary work will focus on testing the feasibility of studying transplacental exposures using questionnaire-type epidemiologic data.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: Many environmental exposures that potentially increase human disease risk have not been well documented due to the lack of appropriate methods for study. The development of new study techniques and the refinement and testing of other more standard methods of study will enable the epidemiology program to address some of these less well-studied areas. A unique aspect of the proposed course of the project is the anticipated coordination of laboratory and population research techniques.

PUBLICATIONS

Brown, S.M.: The use of epidemiologic data in the assessment of cancer risk. J. Environ. Pathol. Toxicol. 4(2,3): 573-580, 1980.

Everson, R.B.: Individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life. The Lancet, July 19, 1980, 123-127.

Rogan, W.J.: Analytical chemistry needs for environmental epidemiology. In McKinney, J. (Ed).): Environmental Health Chemistry. Ann Arbor, Ann Arbor Science Publishers, 1980, 123-34.

Rogan, W.J.: The sources and route of childhood chemical exposures. J. Pediatr. 97: 861-865, 1980.

Rogan, W.J., and Rall, D.P.: The National Toxicology Program and the pediatrician. J. Pediatr. 97: 79-80, 1980.

Sandler, D.P., Matanoski, G.M., Comstock, G.W.: Health consequences of nasopharyngeal radium exposure. In Proceedings of the Symposium on Biologic Effects, Imaging Techniques and Dosimetry of Ionizing Radiations, June 1979. HHS Pub (FDA) 80-8126, July 1980.

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.

Rogan, W.J.: Environmental and occupational medicine. In Arnold, C. and Kuller, L. (Eds.): Progress in Preventive Medicine, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43007-02 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Detection of Human Exposure to Mutagenic Substances by Analysis of Body Fluids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard B. Everson Epidemiologist EB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is to develop methods for detecting mutagenic substances in human body fluids and to use these methods in studies of environmental epidemiology. Laboratory methods necessary for these studies are currently being investigated and model populations studied. Following clarification of technical issues in these studies, body fluids from individuals suspected to have occupational or other environmental exposure to mutagenic substances will be analyzed to confirm and partially quantitate such exposure.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: This study involves two overlaping phases: assay development and human studies. Assay development will adapt short term mutagenesis assays to the measurement of mutagenic substances in body fluids. Such adaptation will include investigation of (1) methods for sample extraction, concentration and deconjugation; (2) sensitivity of these assays and their response to interactions between elements of complex biologic mixture; (3) possible technical artifacts and statistical interpretation of results. Initial work will focus on using the Salmonella Plate Assay, but the efficacy of other endpoints such as 8-azaguanine resistence in bacteria will be investigated. In some instances chemical determinations for specific substances will be employed so that these determinations can be correlated with mutagenesis experiments. In addition, investigation of the most effective methods for timing, collection, storing, and processing samples from human subjects will be investigated by analysis of urine from patients undergoing chemotherapy for malignant diseases. Results from these investigations will be used in the design and interpretation of studies or other human populations.

MAJOR FINDINGS AND PROPOSED COURSE: Procedures for calibrating counts from automated colony counters have been investigated statistically, including effective counting procedures for high density plates. The effect of the histidine present in body fluids on numbers of spontaneous revertants in the Salmonella Plate Assay has been explored, and this information applied in interpreting results from previous assays of body fluids. Currently, protocols for human studies and for collaborative studies of human populations are being initiated and undertaken.

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 of human exposure to mutagenic substances.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43008-02 EB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Use of the Laboratory in Studies of Environmental Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard B. Everson	Epidemiologist	EB	NIEHS
Other:	Thomas Wong	Staff Fellow	EB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	1.4	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long term objective of this project is the effective use of the laboratory in studies of environmental epidemiology. It will include interdisciplinary development of ideas and methodologies, coupled with attention to details of both the laboratory procedures and the gathering and analysis of human data. The effort will focus on the development of techniques identifying possible genetic damage in man by identification of exposures to genetic toxins or disruption of human cellular material suggesting genetic damage.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED AND PROPOSED COURSE: To encourage an interdisciplinary approach to studies of human disease etiology, especially disease related to genetic toxicology, a laboratory unit has been established within the Epidemiology Program of the Biometry Branch. Specific ongoing projects include detection of alterations in mixed function oxidase enzymatic activity associated with tissue and body fluid levels of organochlorine pollutants (Z01 ES 43002-05 BB) and development and use of assays to detect mutagenic substances in body fluids (Z01 ES 43007-02 BB). Planned studies include investigations of assays that could serve as potential indicators for genetic damage in man, and the effective use of these assays in occupational and other studies related to environmental epidemiology.

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 determine outcomes 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44003-04 EB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Allen Wilcox	Medical Officer (Research)	EB NIEHS
Other:	Beth C. Gladen	Statistician	SBB NIEHS
	Dale Sandler	Staff Fellow	EB NIEHS

COOPERATING UNITS (if any)
Statistics and Biomathematics Branch,
Environmental Epidemiology Branch, National Cancer Institute

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a long-range project designed to improve epidemiologic methods for studying reproductive outcomes, particularly for studying birthweight and spontaneous abortion, and to investigate the association between environmental exposures and reproductive outcomes. There are several facets to this project. A study has been designed to test the validity of recalled reproductive data obtained by questionnaire. The basic epidemiology of spontaneous abortion is being investigated using an existing previously-unanalyzed data set. New methods for analyzing environmental effects on birthweight and fetal growth are being developed. Finally, a prospective study of early fetal loss, using recently-developed pregnancy tests, is being planned.

PHS-6040
(Rev. 2-81)

PROJECT DESCRIPTION

METHODS EMPLOYED: Reproductive outcomes are a sensitive endpoint for the detection of human exposure to environmental hazards. The purpose of this project is to develop sound epidemiologic methods for measuring human reproductive outcomes, and to clarify the relationship of those outcomes to particular exposures. In particular, effort has focused on (1) the development of new methods for analyzing the effect of environmental hazards on birthweight and fetal growth; (2) a description of the epidemiology of spontaneous abortion; (3) a test of the validity of recall data regarding prior spontaneous abortion; (4) the design of a prospective study of subclinical early fetal loss, using newly-developed assays for human chorionic gonadotropin.

MAJOR FINDINGS AND PROPOSED COURSE: (1) We have shown that certain commonly-accepted methods for analyzing birthweight and fetal growth are unsound. Current efforts are directed to refine and extend fundamentally new analytic methods for detecting environmental influences on birthweight. (2) The descriptive epidemiology of spontaneous abortion has continued, with a new expoloration of the relation of spontaneous abortion risk to age at menarche and gravidity, and an estimation of the distribution of inherent risk of abortion among women. (3) Data regarding the validity of reproductive questionnaire responses should be available for analysis by the end of this fiscal year. (4) The proposed study of early fetal loss is being prepared for peer review, and is scheduled to begin in the next fiscal year.

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 not well-understood, and are persistently difficult to measure, and to analyze. This project intends to strengthen the epidemiologic tools for measuring spontaneous abortion and for analyzing birthweight and by so doing, to better assess the contribution of environmental factors to human reproductive loss.

PUBLICATIONS

Wilcox, A.J.: Birthweight, gestation and the fetal growth curve. Am. J. Obstet. Gynecol. 139: 863, 1981.

Wilcox, A.J., Treloar, A.E., and Sandler, D.P.: Spontaneous abortion over time: Comparing occurrence in two cohorts a generation apart. Am. J. Epidemiol., in press.

COMPUTER TECHNOLOGY BRANCH

COMPUTER TECHNOLOGY BRANCH
Summary Statement

The Computer Technology Branch has the responsibility of providing computing and data processing support to NIEHS and the National Toxicology Program. This service may be thought of as consisting of three cooperating and interdependent efforts, namely computer operations and support programming, information systems development and computer engineering.

The computer operations and programming effort assumes the responsibility for maintaining NIEHS' PDP 11/70 computer system and a network of terminals connected to the various computers at NIH/DCRT, 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 Comparative Medicine Branch. For the NTP, projects underway include the Environmental Mutagenesis Information System, the Carcinogenesis Bioassay Data System, the Toxicology Data Management System (in cooperation with the National Center for Toxicological Research), and the Chemical Information and Tracking System.

Provision of computer engineering support to the laboratories of the Institute is also ongoing within the Computer Technology Branch. Solutions are being sought to engineering problems related to all aspects of computer hardware and software. Tasks within this effort have included the specification of minicomputers, peripherals, and vendor-supplied software; the design of timing devices and interfaces between minicomputers and laboratory instruments; and the development of software for control of experiments, data acquisition, data analysis, and data transfer.

LAWRENCE BERKELEY LABORATORY/UNIVERSITY OF CALIFORNIA
Berkeley, California 94720
(222Y01-ES-10066)

TITLE: Quantitative Species Extrapolation in Carcinogenesis

CONTRACTOR'S PROJECT DIRECTOR: Bruce N. Ames, Ph.D.

PROJECT OFFICERS (NIEHS): M. D. Hogan, Ph.D. and
J. E. Huff, Ph.D.

COLLABORATING INSTITUTE: Department of Energy

DATE CONTRACT INITIATED: April 1, 1981

CURRENT ANNUAL LEVEL: $187,500

PROJECT DESCRIPTION

OBJECTIVES: The objective of this research effort is to construct a large-scale, computerized data base employing information abstracted from existing research reports of animal cancer bioassays and to use this data base to investigate a variety of issues that are relevant to the general problem of quantitative species extrapolation in carcinogenesis. These issues include: (1) the similarity or lack of similarity of potency indices calculated from independent tests on the same compound, (2) the comparability of results for males and females from the same species and strain, (3) the degree of correlation in the responses of different strains within the same species, (4) the comparability of rats and mice with respect to both overall sensitivity and preferred target organ, (5) the comparability of rodents and other species, particularly long-lived species such as primates, (6) the comparison of potency indices for the same chemical administered by different routes or dosing schedules, and (7) the comparison of potency indices for lifetime and less than lifetime exposure.

METHODS EMPLOYED: A set of acceptability criteria for determining which studies reported in the carcinogenic literature should be abstracted into the computerized data base has been established. A computerized data file using information from studies that conform to these acceptability criteria is now being constructed. Initially, data base construction will focus on the NCI Bioassay Data generated through 1978 and the more than 150 other studies on chemicals tested by NCI that have been reported in the literature. Statistical computer programs have also been developed for calculating relative potency indices using information from this data file in order to address the various issues outlined in the above stated objectives.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Given our increasing reliance on laboratory animal data to generate quantitative 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.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

During Fiscal Year 1981, the National Advisory Environmental Health Sciences Council reviewed 442 applications assigned to NIEHS as primary or secondary assignee. This represents an overall decrease of 46 applications from Fiscal Year 1980. One hundred and twenty-eight new and competing awards were made: 106 regular research grants, two Environmental Health Sciences (EHS) Centers (competing), two Research Career Development Awards; four Mid-Career Development Awards, and 12 Individual NRSAs and two Senior Fellowships. No new or competing applications were funded in the Marine and Freshwater Biomedical (MFB) Center program. These new and competing awards, plus the non-competing awards, brought the 1981 total awards to 406 active grants, a decrease of 17 awards from Fiscal Year 1980.

The New Investigator Research Award (NIRA), previously the Young Environmental Health Scientist (YES) Program, has continued to attract young investigators although a lower level (28 active awards) than in previous years. The decline in number of NIRA applications and R01 applications reinforces the decision recently made to issue RFAs in special emphasis areas and to begin a systematic review of the current research portfolio in anticipation of developing program announcements in select areas where the number of applications are decreasing or in areas earlier identified by the Task Force and by Council for increased emphasis.

Requests for applications (RFAs) have been issued or are being developed for issuance in FY 81 in "Extrapolation and Risk Estimation"; "Human Health Effects of PBB"; "Immunotoxicology to Environmental Agents; and "Biological Effects of Chemical Interactions".

Preliminary discussions have been initiated with staff of National Institute of Occupational Safety and Health (NIOSH) to explore joint issuance and possible co-funding of a program announcement in reproductive toxicology research. This program, if approved, would become active in FY 82.

A new position as Chief of Program Analysis and Scientific Review was created and filled in FY 81. Computerization of EP grants is progressing. The scientific vocabulary has been placed on the computer and will be used with IMPAC to complete automation of the grant program, at least on an interim basis while other, possibly more responsive, systems are being explored.

The Secretary, DHHS, approved the charter for an Environmental Health Sciences Review Committee. Potential members are being identified and all administrative activities are being completed pending approval to appoint members. Once the Committee is in place, NIEHS will assume responsibility for review of Program Projects in addition to its current grant review responsibilities.

The EP has also assumed responsibility for review of NIEHS funded contracts. Dr. John Braun recently joined EP and is developing EP review guidelines.

RESEARCH MANPOWER DEVELOPMENT CENTER

Training Programs

The NIEHS Extramural Training Programs support pre- and postdoctoral research training in the fields of environmental toxicology, environmental pathology, environmental epidemiology and biostatistics, and environmental mutagenesis. Each of these programs has as its goal to provide appropriately trained manpower for careers in research pursuant to the missions of the Institute. Program descriptions and summaries are provided in the following text.

Environmental Toxicology

Training in environmental toxicology is focused on developing individuals for research careers pursuing an understanding of the pharmacological principles which determine the mechanism, site, and severity of damage to tissues by environmental agents. Research training focuses upon the processes by which environmental agents affect biological systems and by which biological systems influence the action and fate of these agents (metabolism, tissue distribution, etc.). Approximately 65% of our training support dollars are spent in the area of environmental toxicology.

To better define "environmental toxicology" let us divide working toxicologists into the following classes:

1. Clinical Toxicologists who deal with acute intoxication by poisons. These individuals determine causes of intoxication (which chemical?) and prescribe methods of treatment. They are usually associated with hospitals or poison control centers.

2. Experimental Toxicologists who are involved in the demonstration of safety or hazard of exposure to chemical or physical agents such as environmental chemicals or drugs. These individuals do testing on experimental models. They design protocols and analyze results. Their work usually culminates with a "safety evaluation" report including protocol descriptions and the data obtained in support of a conclusion on hazard level resulting from exposure to the particular agent.

3. Predictive Toxicologists who speculate on the hazard to man from exposure to chemical or physical agents: These individuals attempt to obtain knowledge about the pharmacodynamics and pharmacokinetics, mechanisms of action, dose/response relationships, comparative metabolism, etc. of the particular chemical involved. They do research on experimental models in an attempt to extrapolate this knowledge to better assess the hazards to man of exposure to the chemical.

4. Forensic Toxicologists who determine cause/effect relationships between poisons and pathologies: These individuals usually work with law enforcement agencies.

The NIEHS supports the training of pre- and postdoctoral students in the second and third classes of toxicologists, focusing on those individuals who wish to study the effects of environmental agents.

There are generally two schools of thought regarding how individuals should be trained for research careers in environmental toxicology. The philosophy of the first school is that a student must initially be trained as a scientist in a classical discipline and then receive additional training and experience to specialize in toxicology. This has been the general pathway by which today's established toxicologists have moved into the field. Individuals in this school argue that toxicologists must first be trained as good basic scientists so that the scientific method can be applied in well-defined experiments to determine the nature of the toxicity at the biochemical, physiological, cellular or tissue level.

The philosophy of the second school is that toxicology is a discipline unto itself which draws skills and backgrounds from various facets of the basic sciences. Therefore, a toxicologist should be trained not only by taking courses in toxicology and in special topics which emphasize the interaction of environmental chemicals with the biochemical systems, organelles, macromolecules, or the tissue component.

The NIEHS Extramural Training Program in Toxicology supports institutions training individuals under both these philosophies. Therefore, our award portfolio is comprised of grantees in a variety of departments such as biochemistry, physiology, nutrition and food sciences, etc., in addition to Departments of Toxicology.

It is becoming quite apparent that there are needs for toxicological specialists to engage in two types of activities. The immediate need is for toxicologists to perform safety evaluations to provide data required by the regulatory agencies upon which societal decisions can be made as to whether or not the general population should be exposed to a certain material. These are the experimental toxicologists referred to above. Likewise, there is an equal demand for individuals who will advance the state-of-the-art of the science of toxicology by furthering our understanding of toxicological mechanisms of action by developing new testing methodologies and by producing new knowledge about the effects of chemicals on organisms. This new knowledge will then allow safety evaluation procedures to become more precise and more capable of predicting the true hazards of these agents in the real world. (Figure 1 shows how the training of toxicologists may differ beginning at the postdoctoral level depending on the choice of career pathway.)

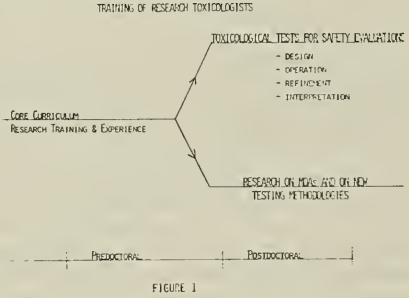

FIGURE 1

The demand for individuals completing their training in both these specialities of toxicological sciences is rapidly expanding. The demand for individuals capable of performing research in safety evaluation comes from all sectors of the economy. At least two recent surveys on the capacities of safety evaluation laboratories have concluded that the demand for such services in the next few years will far exceed the capacity of these laboratories to absorb the work. The limiting factor is the availability of trained individuals to design, conduct and interpret the testing protocols.

The demand for research toxicologists also comes from academia, government and private research laboratories and, in the long run, it is the activities of this group of people which will bring us out of the quagmire of uncertainty regarding how to do proper safety evaluation.

Grants for institutional support of new and extant training programs in environmental toxicology made during the past year are described below:

The Massachusetts Institute of Technology, Department of Nutrition and Food Science has a training grant to support pre- and postdoctoral students for training in biochemical, chemical and biological approaches concerned with problems relating to the public health impact of environmental chemicals. Specific thrusts are the identification of toxic, carcinogenic and mutagenic methods for their detection and quantification; the development of model systems for the study of their formation; the characterization of their acute and chronic toxicity, carcinogenicity and mutagenicity; the study of their metabolic activation and inactivation and attempts to elucidate their mechanisms of action.

The University of Rochester, School of Medicine and Dentistry, Department of Pharmacology and Toxicology has an award to provide pre- and postdoctoral training in environmental toxicology in a broad range of major problems such as behavioral, biochemical, developmental, and inhalation toxicology. Trainees select preceptors and thesis topics from the faculty of the Environmental Health Sciences Center at that institution.

The training program at Vanderbilt University prepares pre- and postdoctoral trainees for careers in toxicology by emphasizing a chemical and metabolic approach to the structure, mode of action, and the mechanism of action or deactivation of a variety of chemicals found in the environment. Training emphasizes studies on metals, industrial toxicants, pesticides, mycotoxins, and other naturally occurring toxicants.

A grant to the Pulmonary Medicine Division of the Duke University Medical Center supports the training of Ph.D. and postdoctoral scientists in environmental problems of the human lung. The focus of this training grant is on the defense mechanisms of the lung after exposure to environmental agents such as silicon dusts and pyrolized hydrocarbon particulates. The program is multidisciplinary utilizing faculty and facilities from several university departments.

The research training for postdoctoral and advanced predoctoral students at the Medical College of Wisconsin in environmental toxicology focuses on investigations of basic mechanisms of toxicant action. The program provides a mix between applied toxicology and basic toxicological principles with didactic training in biochemistry, physiology, pharmacology, and toxicology.

Research activites emphasize environmental toxicology, aquatic toxicology, human toxicology, chronic toxicology, and systemic toxicology.

A training program at the University of Mississippi Medical Center in the Department of Pharmacology and Toxicology provides training in modern toxicological sciences, basic medical sciences, and pharmacological, pathological, and biochemical considerations which are related to the evaluation of the safety of toxic materials. The program is oriented toward a metabolic approach to the evaluation of the toxicity of chemicals which are found in the environment. Examples of such chemicals are food additives, industrial toxicants, agricultural chemicals, air and water pollutants, and mycotoxins. Trainees receive instruction in all phases of toxicology including enzymology, isotope techniques, isolation and identification of metabolic products, and problems related to the comparative evaluation of animal species and man.

A program in Environmental Toxicology and Physiology at Yale University training postdoctorals is being supported. Students receive research training in the area of effects of toxicants on lung and liver function in developing animals, mechanisms of action of air pollutants on airway resistance, the basic pharmacology/physiology of airway passage smooth muscle, and the cardiovascular responses to heat exposures. These experiences will prepare individuals for careers in research and teaching in the areas of environmental toxicology, environmental pathophysiology, or environmental physiology.

A multidisciplinary training program in toxicology is supported at North Carolina State University in the Department of Entomology. Trainees select from emphasis areas such as biochemistry, entomology, botany, and zoology. Trainees receive instruction and research guidance from faculty members in these departments as well as from interactions with appropriate individuals in the laboratories of the NIEHS, the Environmental Protection Agency (EPA), and several private laboratories in the geographic area of this institution.

A predoctoral training program at the Children's Hospital Research Foundation in Cincinnati provides trainees with a rigorous foundation for research careers in teratology. Training in molecular teratology is carried out through the interdisciplinary graduate program in development biology at the University of Cincinnati, which awards the Ph.D. degree. Students approach the subject of teratology as an abnormal manifestation of development. The molecular aspects of this subject are emphasized and the mechanism by which environmental agents cause molecular perturbations in development and lead to physiologic, morphologic, and functional deficits is studied. The role of pharmacodynamics and biotransformation of environmental agents is stressed.

The Department of Entomology at Cornell University has a training grant to provide predoctoral and postdoctoral training in several disciplines falling within the field of environmental toxicology. Primary focus is on the molecular interactions of toxic materials such as drugs, pesticides, industrial pollutants, and natural products with living organisms and the environment. Specific thrusts are in the areas of neuropharmacology, mechanisms of action of toxicants, metabolic degradation of environmental agents and assessment of toxicological hazard to man from these materials.

A grant emphasizing predoctoral training to the Department of Environmental Toxicology at the University of California, Davis stresses the chemical aspects

of the science. Physiological and toxicological consequences of low-level exposure to toxicants are determined by the use of biochemical or behavioral endpoints. Structure-activity relationships to allow prediction of physiological distribution and of likely molecular interactions are sought. Metabolic differences between species are studied to allow better prediction of hazard to man through the use of experimental laboratory models for the assessment. Finally, natural toxicants in the human food supply are identified and risk assessments made.

A training program in toxicology at the pre- and postdoctoral levels is being supported at the University of Kansas Medical Center, Department of Pharmacology. The predoctoral training in environmental toxicology provides experience with problems of risk evaluation and problems of standard setting. A complete curriculum of course and laboratory work is followed by laboratory rotations and finally a research project. Postdoctoral students may take appropriate courses during their research trainee experience. The primary focus of research training on this grant is in the area of biochemical or behavioral toxicology.

The primary purpose of the training program at New York University is to train individuals for research careers in inhalation toxicology, including data evaluation and interpretation. Students learn to plan, conduct and interpret inhalation and other toxicological studies appropriate to the environmental health issue in question. Courses in the basic medical sciences, environmental health sciences, inhalation chamber technology, and the physical and physiological behavior of gases and aerosols are provided in addition to relevant research experience.

An award to the Johns Hopkins University School of Hygiene and Public Health, Department of Environment Health Sciences supports pre- and postdoctoral students learning to evaluate and predict the effects of environmental pollutants on biological systems. Faculty from a number of biological specialities including pharmacology, physiology, biochemistry, biophysics, immunology, genetics and cellular and molecular biology participate in a program focused on learning the basic interactions of environmental chemicals with biological systems. Specific research interests of the staff include hepatic and pulmonary toxicology, neuro and behavioral toxicology, inhalation toxicology, pharmacokinetics, developmental toxicology, mutagenesis, carcinogenesis, and the influence of genetic abnormalities on the response to environmental chemicals.

A Ph.D. program in environmental toxicology in the Department of Environmental Health at the University of Cincinnati College of Medicine consists of an intensive core curriculum followed by specialization in environmental carcinogenesis, mutagenesis, teratogenesis, or neurobehavioral toxicology.

A training program in biophysics at the Roswell Park Memorial Institute trains pre- and postdoctoral students to study the effects of environmental agents on biological systems at the molecular level. Specifically, changes in biomolecular structures caused by agents such as chemical carcinogens, ionizing radiation, ultraviolet radiation, and repair inhibitors are stressed.

A grant to the Institute for Environmental Studies at the University of Illinois supports pre- and postdoctoral trainees in the areas of pharmacokinetics of toxic substances, detection and characterization of micropollutants, comparative

toxicology, study of biocides for selective toxicology, and studies of the movement of toxic chemicals through the environment.

Postdoctoral training for the development of independent research scientists in the area of environmental carcinogenesis and mutagenesis is being provided with NIEHS support at the College of Osteopathic Medicine at Michigan State University. In addition to receiving intense research experience, students are expected to acquire a broad overview and basic understanding of the major problems in the area of environmental biology. The concentration of this grant is on the molecular events which influence the formation of tumors and cancer development.

An award to Duke University Medical Center, Department of Physiology and Pharmacology trains postdoctoral students in the biochemical, physiological and pharmacological aspects of environmental toxicology. Specific areas of emphasis are enzymology, cell biophysics, and integrative physiology.

At Oregon State University a program for both pre- and postdoctoral students in environmental toxicology is being supported. Research training involves the study of a diverse group of environmental toxicants, both natural and synthetic at different levels of biological organization. The program includes training in biochemistry, food science, pharmacology and toxicology nutrition, and pathology. Upon completion of the program, trainees will have developed a level of expertise in problems of environmental toxicology relating to human health and will be able to conduct research in such areas as the determination and quantitative assessment of detrimental effects of environmental chemicals, their modes of actions, and their fates and metabolisms.

The Center for Environmental Toxicology at the University of Wisconsin is supported to provide postdoctoral training in general environmental toxicology. Examples of special research training areas at this institution are mechanisms of action of chlorinated hydrocarbons on mammalian tissues and on postdefense mechanisms, identification and metabolism of mycotoxins, carcinogenesis determination, solvent toxicity, pesticide toxicity, and the nature, persistence and toxicity of organic chemical byproducts.

A program at the Albany Medical College is strongly committed to training students in the area of evaluation of toxic substances. Students are taught to utilize the various disciplines or subjects in the program to analyze and solve difficult and knotty problems in toxicology. Graduates should be able to delineate the best approach to the evaluation of safety of any specific compound to observe and interpret the manifestations of toxicity and to devise and implement programs aimed at elucidating the mechanisms of toxic actions.

A program in environmental toxicology at Purdue University is being supported and utilizes an interdisciplinary approach to toxicology training. The disciplines of bionucleonics (radionuclides), veterinary pathology, entomology and pharmacology/toxicology are all potential facets of this program. Biochemical mechanisms of toxicity are stressed so that individuals trained in the program have sound scientific backgrounds on which to base and interpret health hazard assessment experiments.

In addition to these institutional awards, some 30 individual postdoctoral fellowships in toxicological sciences are being supported. The majority of these are giving training to individuals pursuing careers in research in

toxicology with emphasis on materials such as heavy metals and ambient organic chemicals. Four of these awards are for training in behavioral toxicology and two are for research training in ecotoxicology.

Environmental Pathology

Trainees in this area focus their research on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees hold professional or academic degrees which qualify them for advanced training in gross and histopathological research dealing with the structural and functional alterations of tissues exposed to environmental chemicals. Usually training is focused on morphology, but training in the biochemical basis of environmental disease pathogenesis may also be included in these programs and the tools of the toxicologist can also be used to elucidate the mechanistic bases of environmental diseases.

Figure 2 illustrates the difference in thrusts between environmental pathology and environmental toxicology training.

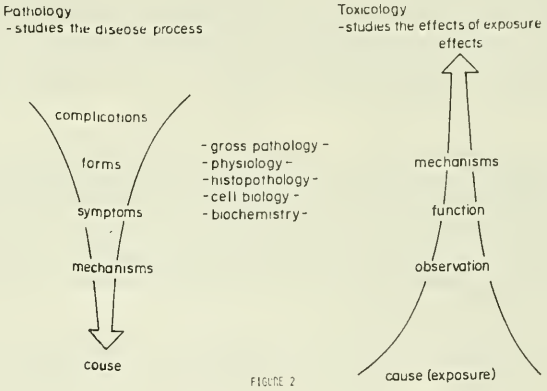

FIGURE 2

The objective of the pathologist is to determine the cause of the observed lesion, whereas the objective of the toxicologist is to assess the effects of exposure to a known agent. Nevertheless, the tools used by both disciplines in the various facets of investigation may be identical. Therefore, graduates of the environmental pathology training programs can become members of research teams involved in chemical risk evaluation utilizing laboratory animals as experimental models. Although these graduates are capable of histopathological evaluation, their backgrounds allow them to become more active members of research teams involved in chemical risk evaluation or to become independent investigators.

Four institutional awards for training in environmental pathology, described below, comprise the current program.

The environmental pathology training program at the University of North Carolina School of Medicine provides postdoctoral training in experimental environmental pathology and human comparative pathology of diseases and lesions produced by chemical and physical contaminants in the environment. Postdoctoral trainees work in an apprenticeship relationship with senior scientists on research involving the pathogenic effects of environmental contaminants such as heavy metals, carcinogens, and mutagens particularly as they relate to DNA replication and repair, abnormalities of hemostasis, and cell and tissue ultrastructural effects.

The environmental pathology program at the School of Veterinary Medicine at the University of California, Davis allows postdoctoral trainees 12-18 months to take advanced courses appropriate to pathology and environmental toxicology and thereby to become familiar with environmental health problems and research needs. The training emphasizes observation of morphological abnormalities resulting from exposure to environmental agents, and interpretation of these abnormalities in a manner which generates new knowledge about their effects. Neuropathology, pulmonary pathology, teratology, and developmental immunology are stressed in this program.

The Department of Pathology at the University of Washington School of Medicine likewise trains in the morphological manifestations of exposures to environmental agents. A broadly-based training faculty in the related fields of toxicology, pathobiology, teratology and behavioral toxicology participate in training individuals to have strong capacities to investigate the cellular and subcellular effects of exposure to contaminants on the nervous, respiratory, renal and gasto-intestinal systems. Likewise, the effects of congenital exposures to environmental agents on brain structure and behavior as well as pathological studies are pursued.

The Washington University (St. Louis) School of Medicine, Department of Pathology received support for training pre- and postdoctoral students in the areas of environmental carcinogenesis, the effects of environmental agents of the developing nervous system, the relationship between air pollution and chronic diseases such as emphysema, hypertension and arteriosclerosis and on basic mechanisms in cell injury and death induced by environmental agents.

A program in environmental pathology of the respiratory system for pre- and postdoctoral students is underway at the State University of New York at Stony Brook. An interdisciplinary curriculum in structure and function plus laboratory rotations prepare students for research projects primarily dealing with the effects of contaminants in the lung. The program goal is to prepare scientists to independently design, conduct, and evaluate investigations of the impact of environmental agents on the respiratory organs.

Environmental Epidemiology and Biostatistics

Trainees in environmental epidemiology are taught to utilize statistical and mathematical tools to assist in the identification of environmental diseases in human populations. Training stresses non-infectious disease epidemiology with emphasis on the identification of causes of environmental diseases. The major difference between training in environmental epidemiology and infectious disease

epidemiology is that human populations are studied with respect to the effects of established exposures to environmental agents, whereas epidemiological studies of infectious diseases usually begin with a pathological observation.

Biostatistics trainees learn and apply mathematical and statistical tools in assisting environmental health scientists in experimental design and interpretation. Mathematical modeling for human risk assessment based on laboratory experiments is studied. Methodologies for extrapolating the results of high dose exposures to environmental agents to predicted effects or real-world low dose exposures are also developed. Upon completion of their training, these individuals undertake research activities supportive of the development of laws and standards governing human exposures to environmental agents.

In addition to one postdoctoral fellowship in epidemiology and one in biostatistics, current support for institutional training was as follows:

A grant to Harvard University School of Public Health, Department of Epidemiology trains pre- and postdoctoral students for research investigations on the effects of environmental chemicals on humans. A combination of courses in environmental health, biostatistics, epidemiology, toxicology and occupational health preceeds research activities in this field. Postdoctoral students (usally post M.D.) also receive a Master of Science in Epidemiology from this program.

The University of North Carolina (UNC) School of Public Health, Department of Epidemiology likewise trains students in the principles and perspectives of epidemiology and biostatistics as well as environmental sciences and human biology to develop a capability to initiate epidemiological inquiries to test hypotheses on the biological effects of environmental agents. Subsequent to didactic training, a research problem is selected drawing on the resources of the University, the Environmental Protection Agency, the National Institute of Environmental Health Sciences and the Occupational Health Studies group at the UNC.

A program at the University of North Carolina in biostatistics emphasizes the mathematical aspects of research in environmental health studies and the provision of statistical support to workers in the field. Trainees participate in research problems on measurement of individual environmental hazards, interactions of several hazards, measurement of doses and responses in human populations and estimation of dose-response relationships.

A program to train Ph.D.'s in epidemiology and statistics related to environmental health at Yale University is being supported. Courses in statistics and epidemiology are combined with appropriate didactics in biology (e.g., pathology and bioassay) and are followed by dissertation research in specific areas of environmental health.

A pre- and postdoctoral program to train environmental epidemiologists and biostatisticians is being supported at the University of Cincinnati College of Medicine. The objective of the program is to train individuals skilled at determining untoward consequences of environmental agents which affect the health of all members of society. Ongoing studies include epidemiological studies of environmental factors and disease such as drinking water and cancer rates, pesticides and their health effects, the health effects of lead pollution

to residents living near smelters and exposure to ionizing radiation and thyroid disease. In addition, training is given to individuals who provide epidemiological and biostatistical support to a wide variety of studies in environmental health.

A pre- and postdoctoral training program in environmental epidemiology at the University of California, Los Angeles stresses industrial, agricultural, and urban environmental studies. The program attempts to train environmentally oriented epidemiologists and biostatisticians to study low level chronic exposures to environmental pollutants and their effects.

Environmental Mutagenesis

Trainees in this area are taught to apply the basic principles of genetics to applied studies aimed at assessing the potential genetic hazard to man of environmental chemicals. Training emphasizes: (1) the understanding of chemical factors which predict a compound's ability to alter the genetic makeup of man, (2) the development of reliable test systems with unequivocal quantitative relevance to man for detection and quantification of mutations in germinal cells, and (3) the elucidation of molecular and cellular mechanisms in mutagenesis.

At the University of California, Berkeley a program supporting both pre- and postdoctoral students for research training in environmental mutagenesis, carcinogenesis, and teratogenesis is being supported. Research training is provided in related areas of biochemistry, biophysics, and biophysical chemistry. Cellular mechanisms are stressed.

This is the smallest area of NIEHS training support and is comprised of the aforementioned institutional award and minor parts of several of the awards to institutions for training in toxicology.

Institute of Environmental Medicine
New York University Medical Center

Major activities are highlighted in six programs.

1. Toxicology. Discriminative function related to age has been tested in animals and in humans. Normal human adults and children have volunteered to perform the computer-controlled test of accuracy of discriminating visual shapes and time duration. By determining the degree of correlation between test results from normal animals and humans, the validity of using animal tests for predicting the behavior of humans has been established.

Neurotoxicity of acrylamide in pigeons and in mice was tested. The battery of tests used included food intake, visual and motor coordination, hind limb grip strength, and complex discriminative behavior. All of the behavioral tests documented early effects of acrylamide before signs such as ataxia and loss of body weight were observed.

New assays for neurotoxicity induced by organic solvents are being tested. Mice, pigeons and macaque monkeys have been chosen as models for human systems of appetite change and sleep disturbances which result from sub-chronic inhalation of benzene, toluene, trichloroethylene or hexane. Some of the new assays being investigated are sufficiently streamlined to permit their use in routine toxicity screening.

Studies were continued on the metabolism and toxicity of sulfite and sulfur dioxide in mammals. In these investigations, a sulfite oxidase-deficient rat model has been exploited as a means of generating relatively high systemic sulfite concentrations without the incorporation of unrealistic amounts of sulfite salts into the diet. Sulfite metabolism was characterized in these and in normal rats by monitoring the concentrations of sulfite and of two of its metabolites (S-sulfonate compounds and thiosulfate) in the body tissues and fluids.

Subchronic toxicity experiments of approximately 2 months duration using female rats less than 5 months of age revealed a low (2-3%) incidence of mammary adenocarcinoma in sulfite oxidase-deficient individuals compared to a 0% incidence in individuals with normal amounts of this enzyme. There was no suggestion of a dose-response relationship with respect to systemic sulfite exposure.

Research on acrylonitrile (ACN) has shown that diet is an important factor in acute toxicity of ACN. A diet high in fat, whether complete in lipotropic factors (i.e. methionine, choline and niacin) or not is associated with an increased lethality (i.e. lower LD_{50}) in both male and female rats. This diet was also found to be associated with longer hexobarbital sleeping time and elevated brain and liver glutathione content. The increased toxicity observed after oral dosing was not seen in male rats after inhalation exposure, suggesting that hepatic metabolism is a critical factor in acute toxicity.

Continuing studies on the hematological effects of environmental agents have focused on the development of techniques to measure cell membrane fluidity.

Preliminary results indicate that ozone, various metals, and lipid peroxide decomposition products are capable of altering red cell membrane fluidity, as measured by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene. Benzene studies in progress have focused on the synthesis of pure trans, trans-muconaldehyde for study of its possible role as a metabolite responsible for benzene hematotoxicity.

The formation of superoxide anions by the cell membrane in response to PMA, as an important factor in tumor promotion, has been studied. The oxygen radical response, which is the biological weapon employed by leukocytes to kill bacteria, appears to occur on the cell membrane of a variety of cells. Known inhibitors of tumor promotion - protease inhibitors, dexamethasone, and retinoids - blocked the production of free oxygen radicals.

2. Chemical Carcinogenesis. Understanding the disease called cancer is exceedingly difficult since it is not a single disease. It is a large family of diseases which show a wide range of structural and behavioral similarities and deviations from what is regarded as "normal". The problem is further compounded by the fact that even our understanding of the normal cell is relatively rudimentary. In a very real sense, therefore, the comparisons that are made between the aberrant cancer cell and the normal cell are comparisons between unknowns. As a consequence, the research described below involves basic biological investigation, as well as detailed study of environmental factors such as carcinogens and other factors which initiate or modulate the carcinogenic state.

Investigations into the covalent interaction of trichloroethylene (TCE), an extensively used organic solvent, with cellular macromolecules, are continuing.

Studies have continued on the characterization of the adducts from the *in vitro* reaction of chromatin DNA with DBE in the presence of rat liver microsomes as have studies on the detection of adducts produced by the trapping of epoxides with nucleophiles by examining the reaction of epoxides with the nucleophile 4-nitrothiophenol.

Studies have continued on the carcinogenicity of inhaled, direct-acting alkylating and acylating agents. Groups of rats are being exposed by inhalation to bis(chloromethyl)-ether, β-propiolactone, ethyl chloroformate, dichloroacetyl chloride, propylene oxide, or methyl methanesulfonate. These compounds were selected because they display a wide range of chemical reactivities.

The mutagenic and comutagenic effects of various environmental agents are being studied. Under moderate exposure conditions, where survival is high, it has been found that bisulfite alone is not mutagenic in either eukaryotic (Chinese hamster V79) or prokaryotic (*Escherichia coli*) cells. However, bisulfite does act as a comutagen with ultraviolet irradiation. Bisulfite approximately doubles the mutation frequency in UV-irradiated Chinese hamster V79 cells, and it causes a greater than eightfold increase in Trp^+ revertants in UV-irradiated *E. coli*. The comutagenic effect occurs whether cells are exposed to bisulfite during or immediately following UV-irradiation. Experiments with several strains of *E. coli* having varying DNA repair capacities indicate that excision repair is necessary for the comutagenic effect.

The mechanisms of metal mutagenesis and comutagenesis is being studied. A number of metals such as chromate, arsenic and nickel have been

shown to be human carcinogens. Unlike organic carcinogens, most metals, with the exception of chromate, are not mutagenic in standard microbial assay systems.

This lack of mutagenicity may reflect technical problems in the systems used to measure mutagenesis, since some of the metals are highly toxic. Alternatively, it may be that the carcinogenic metals act by a different mechanism.

To study this problem further, an investigation of the mechanism of chromate mutagenesis in E. coli was undertaken to determine the role, if any, of inducible error-prone DNA repair processes (SOS system). Assays were performed for Trp$^+$ revertants, using the fluctuation test.

In contrast to chromate, arsenite was not mutagenic in either E. coli or Chinese hamster cells. Recent epidemiological evidence shows that arsenite may act as a cocarcinogen rather than as a primary carcinogen. Previous work from this laboratory demonstrated that arsenite can interfere with the repair of UV-induced lesions in E. coli. However, at the highest tolerated dose (1 mM), arsenite appeared to primarily inhibit the mutagenic SOS repair pathway.

Nonhistone nuclear proteins are being investigated in mouse epidermal nuclei. Previously reported attempts to isolate epidermal nuclei have yielded preparations contaminated by cytoplasm and other debris, and it was necessary to develop a procedure for the isolation of a pure nuclear preparation. This was accomplished by homogenizing epidermis in dilute citric acid and filtering the homogenate through a series of microporous nylon mesh filters. Final purification was achieved by banding the nuclei in a Metrizamide density gradient.

Studies of dose-response and dose-time relationships of tumorigenesis are continuing. These studies are being done in mouse and rat skin and in hamster lung. To date, the studies have found that in mouse skin, the binding of benzo(a)pyrene (B(a)P) with epidermal DNA and the initiation stage of carcinogenesis are consistent with a linear non-threshold dose-response relationship.

3. Radiation Carcinogenesis and Dosimetry. Studies in progress in this area include the investigation of ways to improve radiation measurements, the use of model and in vivo systems to study the distribution patterns of radionuclides in organs and in bone, and the study of in vivo radionuclide metabolism.

Studies on a new type of photon detection system which can provide magnitude and positional data for a radioactive source without the use of a high-Z collimator are being conducted.

Research was completed on a model that can be used to calculate the accumulation of uranium in different organs of the human body for different kinds of exposure. The metabolic model derived divides the human body into several different compartments; viz. plasma, red cells, "short-term" bone, "long-term" bone, kidney and urine.

A radon monitor has been developed and tested which is capable of measuring environmental levels of ^{222}Rn without interference from radon daughters. The detector operates with an electrostatic field (electret) that collects daughters as they form and removes them from the sensitive counting volume. Four field units are now under construction which will allow the start of long-term measurements of indoor and outdoor levels of radon.

In addition to studies of "normal" metabolism of curium, the effects of chelation therapy using the Ca or Zn salts of DTPA have been researched to include the determination of efficacy at different dose schedules and possible toxic effects due to trace metal depletion.

In experiments designed to define the distribution and retention of different actinide nuclides in the non-human primate, it was noted that, in units of concentration, the aorta may represent the most significant long-term, soft tissue deposition site in the body.

4. Respiratory Disease and Aerosol Physiology. Studies in progress are designed to determine the factors which affect aerosol deposition, translocation, and clearance from the respiratory tract. The work involves the use of models, both static and dynamic, and in vivo test systems.

A series of studies of aerosol deposition were completed in multiple replicate hollow human airway casts extending from the larynx to 3 mm diameter bronchi in which the intrabronchial patterns of aerosol deposition under steady and simulated inspiratory flows were compared. A preliminary analysis of the data for 3 μm and 8 μm MMAD particles indicates that deposition efficiency is dependent upon the Stokes number and other, still unidentified, factors.

Aerosol probe techniques are being evaluated for the in vivo measurement of airway dimensions. The technique derives airway sizes from the persistence of an aerosol in exhaled air after a period of breath holding. For verification and calibration, the method is being applied to freshly excised lungs of human accident victims. The lungs are characterized by mechanical function tests, then tested with the aerosol probe, and finally sectioned and cast for measurement of alveolar airspace and bronchial airway sizes.

In a coordinate study, the effects of magnetite and coal dusts on the numbers and functions of the rabbits' alveolar macrophages are being examined. The animals are being exposed to low (10 mg/m^3) and high (500 mg/m^3) concentrations of these dusts, with a particle size of approximately 3.5 μm MMAD, for 1-2 hours. The amount of dust retained, as a function of time, after inhalation exposure is being determined by the two non-invasive external measurement techniques cited above, i.e., the measurement of remanent magnetic fields within the thorax and the measurement of the γ-radiation from the neutron-activated particles using collimated scintillation detectors.

5. Epidemiology, Biostatistics, and Biomathematics. The Epidemiology program of the Laboratory of Biostatistics and Epidemiology seeks to relate the exposure of human populations to environmental factors to the incidence of disease and physical or performance impairment, with a view toward the identification and elimination of the causative factors. The laboratory is also concerned with the development of effective procedures for the design, analysis, and interpretation of epidemiologic experimental studies. The Laboratory of Biomathematics formulates mathematical models for exposure to environmental agents and for biological processes. Biomathematics also involves the extrapolation of animal data to humans for use in risk assessment.

Activities include an industry-wide study of about 2,000 workers exposed to bis(chloromethyl)ether (BCME), to determine respiratory cancer mortality. A mortality survey of about 1,300 patients given arsenical therapy for syphilis in

the early 1940's, to assess the relationship between cancer and arsenic at low doses. A similar group treated at about the same time with penicillin served as controls, and a historical cohort study to investigate the effects of cutting-oil exposures on cancer mortality in the bearing industry. The United Automobile Workers Union has provided access to records of workers employed from 1955 to the present in a large Connecticut automobile assembly plant.

6. Environmental Pollution and Ecology. Studies in environmental pollution and ecology cover a variety of research areas ranging from the chemical and radionuclide constituents of air masses to pollution of the aquatic environment. Particular emphasis has been placed upon air pollution and upon the chemical contamination of the Hudson River and the coastal waters into which the river empties.

Studies of N-nitrosamines in particulate matter in the New York City atmosphere have continued. Analyses of the seasonal variations in the concentrations of nitrosamines which have correlated the concentrations to those of vanadium, indicate that oil-burning for space heating is an important source of these compounds. The specific chemical identities of the N-nitrosamines involved have not been established, but clean-up and fraction-collection procedures are being developed. In the meantime, further work has been carried out on the risk evaluation of the N-nitrosamines, where benzo(a) pyrene is being used for comparison.

A study was initiated to develop ways to measure and characterize the acidic fraction of the atmospheric aerosol. The strong acid component in the form of the hydrogen ion (H^+) was measured. In situ measurements of H_2SO_4 and all of the ammonium salts of the atmospheric aerosol were made and the sulfur species were distinguished.

The abundance and distribution of PCBs is being investigated in various planktonic organisms in the Hudson River. Samples of microzooplankton, macrozooplankton, ichthyoplankton and algae have been collected regularly over a 250 km stretch of the river from New York City to Albany. PCB analysis has been carried out, where feasible, for individual species, as well as for hatch samples of plankton.

Research on the distribution and abundance of cadmium (Cd) in blue crabs from the Hudson River has continued. Previous work has shown that the crabs contain substantial amounts of Cd, and that most of the Cd is concentrated in the hepatopancreas. The Cd is stored in a protein-bound form. The protein has a molecular weight of about 10,000 and resembles the metal-binding protein metallothionine found in vertebrates. The synthesis of the Cd-binding protein of crabs can be induced in the laboratory by exposure of the crab to Cd.

Research has continued on the fate of the fallout and/or the reactor-produced radionuclides ^{137}Cs, ^{134}Cs, ^{60}Co, ^{54}Mn, and the naturally-occurring radionuclides ^{40}K, ^{226}Ra, and ^{232}Th. These nuclides have now been measured in 500 samples of Hudson Estuary sediment, water, fish, and aquatic vegetation. Results to date indicate a continued equilibrium trend with time in the ^{137}Cs concentration of surficial sediments, and in the total sediment accumulation of ^{137}Cs the vicinity of the Indian Point reactors. This trend reflects the constant low-level input and removal of reactor and fallout-derived ^{137}Cs.

Center for the Study of the Human Environment
University of Cincinnati

Major activities of this Center are highlighted in the following six programs.

1. Toxic and Essential Metals. The toxicology and metabolism of heavy metals, and their interaction with essential trace metals, has represented one of the main areas of research in the Department of Environmental Health.

In the field of lead research, efforts continued to probe the early effects of exposure in man and experimental animals. The study of metallurgical workers as well as children in high risk urban areas are described and neurobehavioral effects of lead exposure are further considered.

Additional work was carried out on the role played by metallothionein (MT) in Cd toxicology and metabolism. Work was completed on a project designed to test the hypothesis that MT plays a part in Cd metabolism by mediating transport of the metal from liver to kidney. In these studies, CdMT was infused continuously for a period of several days at the extremely low levels likely to be encountered in the poisoned animal; in contrast, earlier studies employed large boluses of MT. Although under present more physiological conditions, plasma levels of MT remained below detectable limits, the preferential accumulation of hepatic metallothionein by the kidney could be confirmed. Negative results were obtained in another project designed to determine whether metallothionein in the intestinal mucosa plays a role in the control of Cd absorption. This project necessitated the development of a technique to measure movement of Cd from the intestinal wall into the body. This step in overall Cd absorption proceeds very slowly, at a rate of only 1-2% of that at which Cd is removed from the lumen.

The recent illustration that dietary methyl mercury produced neurological and developmental abnormalities in children exposed in utero has necessitated a reappraisal of the allowable levels of this compound in food. Indeed, preliminary estimates on the levels of methyl mercury in these cases suggests that the fetus may be more sensitive than was previously thought. A separate study of a fish-eating North American population has further defined for adults the presumably safe methyl mercury levels.

Among other metals under study are nickel and aluminum. Effects of aluminum on pulmonary function required the development of a new analytical method for aluminum in biological tissues. Further metal oriented work was carried out in the study on biological effects of fossil fuel combustion products. The metal composition of stack emissions from coal fired power plants using Eastern or Western coal was compared. Significant differences were observed in stack emission condensates, fly ash samples and the original coals.

2. Pulmonary and Inhalation Toxicology. A study was completed on the effects of aluminum sulfate exposure on pulmonary function in rats. This exposure produced changes in lung physiology, biochemistry, and pathology not seen with exposure to potassium sulfate or sulfuric acid. The lungs of exposed animals increased in weight, primarily due to an increase in extracellular connective tissue. Occurrence of fibrotic changes was also indicated by changes in the volume-pressure curves. Histologically fibrosis was observed especially at the level of terminal and respiratory bronchioles. Further work confirmed the preliminary conclusion that neither K_2SO_4 nor H_2SO_4 produced the same effects as

$Al_2(SO_4)_3$. Clearly, the role of Al in Aluminum sulfate toxicity is of primary importance.

A new project was initiated to determine the health effects of asbestos fibers as a function of fiber length. This required in the first place the design and construction of a complex fiber generation and size classification system. Rats were then treated by intratracheal administration of the asbestos fibers, and fibrotic responses of the lung were measured by determination of proline hydroxylase activity. The asbestos treatment dramatically increased lung wet weight and hydroxyproline content.

3. Clinical and Epidemiological Studies. Clinical and epidemiologic studies include investigations of industrial populations at risk in relation to their environments as well as critical evaluations of selected cases in hospital settings. Populations under study include those exposed to a variety of toxicologic hazards including heavy metals, those affecting the respiratory tract, the skin, the nervous system, reproduction, etc.

Studies of the long term health effects of populations exposed to chemicals in the manufacturing of 2,4,5-T and its' contaminants such as TCDD were continued. A mortality analysis of 121 workers who developed chloracne and other adverse effects from exposure to TCDD in a trichlorophenol process accident has been completed and published. The standardized mortality ratio (SMR) for all causes of death in this group was shown to be 0.69 in 32 deaths observed and 46.41 expected. For categories of malignant neoplasm and circulatory diseases, SMRs were 1.00 and 0.68 respectively. However, because of the small size of the cohort and relatively small number of deaths observed, the results of this mortality analysis cannot be considered conclusive. At the present time, a second mortality analysis is being conducted of the entire population which was exposed to 2,4,5-T and its' contaminants from 1948 to 1969.

A study of airway reactivity of various disorders of inhalation, was actively pursued during the year under review. It appears from the results obtained in the investigation of factors affecting disease severity in asthma that immunological aspects are important in determining the severity of this disease. In general, reactivity of the airways is greater in the more severely afflicted patients than in those suffering only milder asthma. No significant correlations were noted between a disease severity score (DSS), representing six clinical and therapeutic parameters.

An epidemiological study of workers exposed to styrene in the reinforced plastics industry was completed. This study represents a cooperative effort of physicians, industrial hygienists, and epidemiologists. Preliminary results were reviewed previously and full details are now being prepared for publication. The study has assembled useful information on the industrial hygiene status, the use of protective clothing, the value of biological indicators and the identification of specific health effects in a working population exposed to relatively high styrene levels. The major exposure occurs through the lungs, and is correlated with urinary excretion of styrene metabolites.

4. Environmental, Analytical and Safety Research. The Division of Environmental Hygiene and Safety continues to be involved in a variety of basic research efforts in industrial hygiene and air pollution. Collaborative support is provided for many projects which require sampling, analysis, the design and operation of inhalation chambers, and environmental survey work.

In the field of analysis, methods have been adapted for assaying the lead content of small blood samples from children or experimental animals. This forms an essential part of the Lead Program Project Study of childhood exposure to lead. Research on the effects of pulmonary exposure to $Al_2(SO_4)_3$ necessitated the development of a more sensitive and reliable procedure for the estimation of small amounts of Al in biological specimens. The method is based on selective extraction of the quinolate from aqueous solution by chloroform after masking or interfering ions. The conditions of the acid ashing procedure proved to be of critical importance.

Another analytical research project was the development of more sensitive and specific methods for estimation of hydroxyproline; this method is now utilized by investigators interested in collagen formation in tissue culture or in lungs of animals exposed to a variety of pollutants.

The ecological consequences of applying municipal waste water sludge to land was investigated in collaboration with Miami University at Oxford, Ohio. The field study assessed changes in vegetation and populations of insects and meadow voles. Voles were trapped at the end of the study and liver and kidney tissue was analyzed for heavy metals. The results showed that while plant biomass was highest in the fertilized plots and lowest in control plots there were no or only slight differences in plant species and diversity. The highest vole population occurred on the sludge treated plots. Kidneys of these animals had up to ten times more cadmium than did animals from control areas; no detectable uptake of zinc, copper or lead occurred in any tissue, nor did autopsy reveal any pathological alterations in lungs or kidneys.

In another study the toxicity and mutagenicity of surface and ground waters was determined in the neighborhood of an abandoned land fill. The water samples from both wells and a creek contained high concentrations of chlorinated organic chemicals including carbon tetrachloride, chloroform, tetrachloroethylene and hexachlorobicycloheptadiene. Some samples proved moderately to severely toxic to cultured mammalian cells. There was also evidence for some mutagenic activity.

The application of biological testing methods for mutagenic potential of a wide variety of environmental pollutants has become an important aspect of screening studies. In a study of comparative potential toxicity of stack exhaust gas condensates from power plants burning Eastern and Western coal, toxicity in cell culture and *in vitro* mutagenic assays were carried out. Differences were noted in the toxicity of the condensates to cells in tissue culture, but little evidence was obtained for mutagenicity.

The aerosol research continued pursuit of improvements in the sampling devices available for the analysis of air particulates. A unique wind tunnel has been designed and is nearing completion. The laboratory air is drawn through the back of filters; the clean air-flow is then contracted to reach high wind velocities in the test section. An aerosol generator, based on the vibrating orifice design, distributes aerosols of known size throughout the test section. An optical counter is now being adapted for insertion into the wind tunnel so that aerosol flow can be monitored accurately at all times. The system is capable of high sampling efficiency and accurate analysis of a variety of aerosols.

A new study was initiated during the current year on the control of indoor air contaminants. Special emphasis is now placed on the behavior of irritant gases

such as formaldehyde and acrolein, and of particulate matter in tobacco smoke. An allied problem is that of the removal of contaminants by diffusional mechanisms in an enclosed space. Use of regular and low tar cigarettes in these experiments suggests that the irritant concentration of the side stream cigarette smoke possesses little if any relationship to published or advertised tar content of the mainstream smoke.

5. Mutagenesis, Carcinogenesis and Teratogenesis (Genetic Toxicity). A broad spectrum of research is being conducted within this program area. There are three main levels of investigation: 1) the mutagenic, carcinogenic and teratogenic effects of clinical and physical agents in whole animals, 2) effects on cells and explants in culture, 3) the molecular mechanism of action as determined in both intact animals and in vitro. Much of the research focuses on effective use of model biological systems employed and the end points measured, rather than on the effects of specific agents. This approach is necessitated by the diversity of chemical agents currently under evaluation.

The study which was initiated several years ago to identify mutagens in municipal water supplies is continuing. Organic residues in such waters are exceedingly complex and assays of their mutagenic or carcinogenic potential may often be complicated by toxic effects. Progress has been made in developing procedures for identifying the active components of such mixtures by coupling bioassays with chemical fractionation techniques.

Mutagenicity of polychlorinated biphenyl isomers and of polychlorinated dibenzofurans present as PCB contaminants was examined with the Ames test. Other complex organic mixtures also under study are those generated by processes of coal gasification and liquefaction. Thus far, coal gasification particulate and ash byproducts have not been shown to possess any mutagenic potential, nor have water wastes from coal liquefaction. In contrast, three coal liquefaction products, two distillate oils, two liquefaction liquids and one solid residue, as well as a coal gasification tar, do contain mutagens. The observation that the sum of mutagenic contributions of each fraction exceeds the mutagenicity of the whole sample may reflect presence of mutually antagonistic components.

The mechanisms of action of various chemical promoters of carcinogenesis has been given considerable attention. It had been previously shown that such promoters can enhance chemically-induced mutation frequencies in cultured V79 cells. Experiments are now in progress to investigate whether carcinogens, and in particular the polycyclic aromatic hydrocarbons, possess potential as promoting agents, as revealed by their ability to enhance mutation frequencies. Since V79 cells possess no intrinsic enzyme systems capable of metabolizing these hydrocarbons to mutagenic species, the promoting and cancer initiating activities should be clearly resolvable. Encouraging preliminary results have been obtained with this technique. Studies are also in progress on tumor promotion in vivo in relationship to immune responses of the host animal. Studies have been carried out to investigate whether various classes of promoters are active as murine T-lymphocyte mitogens and to determine their effects on other properties of immune cells. Phorbol ester and linear alkane promoting agents were found to be co-mitogenic for both T and B lymphocytes. This co-mitogenic activity correlates well with the relative in vivo tumor promotion activity of these compounds.

6. Neurobehavioral and Neurophysiological Toxicology. Acrylamide is a well-known neurotoxin causing degeneration of nerve fiber axones. A study evaluated the sensitivity and reliability of four different testing procedures for the early detection of neurotoxicity following exposure to one of four known neurotoxins: methylmercury, carbon monoxide, acrylamide and ethylnitrosourea. A number of behavioral measures were altered following acrylamide exposure. These included running wheel activity, splayed leg response, D-amphetamine-induced activity and, to a lesser degree, animal rotation. Changes in drug-induced activity appeared earlier and continued to be observed for longer times than any other measure.

Another study of acrylamide focused on the electrophysiological changes in the primary sensory pathways of the somatosensory and visual systems. Electrophysiological findings were compared with a quantitative and objective method of assessing behavioral impairment. The animals had been prepared for repeated electrophysiological testing by implantation of suitable electrodes. Average evoked potentials and spontaneous locomotor activity were then computed before and during the course of 24 weeks of exposure to acrylamide. Acrylamide was used in concentrations of 0, 50 or 100 ppm; it exerted no effect on body weight of experimental animals. The sensitivity of the behavioral measure of intoxication is displayed by the finding of reduced locomotor activity prior to any other visible signs of intoxication. The effect of the toxin on evoked potentials suggests that its action on the ascending somatosensory system resembles that on other systems. Fibers in the secondary visual pathways appear to be especially sensitive to acrylamide.

A study of the sensory and psychomotor effects of certain hydrocarbon solvents in humans was completed. Many solvents are known to be neurotoxic at high concentrations. Effects of three solvents were therefore evaluated during 30 minute exposures and at concentrations up to 4 times the TLV, but no significant effects were noted. Similar work with high concentrations of automobile exhaust has led to the conclusion that the behavioral effects from this source in rats can probably be attributed to hydrocarbons.

Studies on the teratological effects of dichloromethane (DCM) included the study of behavioral changes in rats following exposure in utero. Functional alterations in the behavioral development in the offspring were indeed observed. It is not possible, however, at this time, to decide whether these changes result from a direct effect of DCM on the fetus, or indirectly from elevated maternal carboxyhemoglobin levels or other changes.

A series of investigations on the pharmacological responsiveness of lead-exposed animals indicated that lead exposure modulates the action of amphetamine on motor and sensory pathways. As a next step in these experiments, the regional uptake of amphetamine is now being measured in the brains of lead-exposed animals, in order to determine the locus of the altered pharmacological response. Work also continued on the behavioral effects of early lead exposure.

Mount Sinai School of Medicine

It has been found that the development of improved bullet (a nylon jacket has been designed by Smith and Wesson) has led to a considerable decrease in lead exposure on firing ranges. Another example of the practical utilization of

studies of the lead research group has been the identification of a new environmental "hazard" in the general population; that of lead poisoning in people using "do-it-yourself" heat guns for the removal of lead containing paint, in renovation of homes.

An opportunity has recently presented itself to investigate the long-term, low-level health effects of fluorine contamination. In 1959, with the construction of the St. Lawrence Seaway, and the availability of associated electric power, two aluminum reduction plants were built close by Massina, New York, the most easterly opposite Cornwall Island in the St. Lawrence River. The plants are owned by Alcoa and Reynolds Aluminum. Operation began in 1959 with considerable fluoride emissions, primarily going in a north easterly direction.

Extensive studies by Canadian agricultural authorities have described botanical effects in areas of the St. Regis Reservation while Krook and his colleagues at Cornell have reported extensive evidence of cattle fluorosis. These changes have been accompanied by important alterations in the economy of the reservations, including loss of much of the dairy farming on Cornwall Island and nearby areas on the southern shores of the St. Lawrence, also in the line of the plant's emissions. Many measurements have been made by Canadian authorities and others who have studied environmental fluorine levels. As a consequence, Mount Sinai has been asked by the Canadian Ministry of Health to investigate the health status of the Mohawk Indian population, to determine whether or not evidence exists that would indicate adverse health effects, to allow appropriate remedial measures to be taken.

The wide presence of nitrosamines in the environment (including food) has raised the question of their potential carcinogenicity in humans, particularly in cancer of the colon/stomach. Their carcinogenicity has been amply demonstrated in a large series of animal studies, with a variety of nitrosamine compounds.

An investigation of the total work force of a large ball bearing plant, where operations date back to the early part of World War II has been launched. Over 1,100 long-term workers are involved. The cohort was established with the cooperation of the United Automobile Workers of America and is being traced through 1979. Death certificates are being obtained for those known to have died, and pathological specimens, clinical data and hospital records are being obtained.

Another cohort study is underway of the work force of a plant in Baltimore, Maryland. This involves approximately 400 operating and maintenance personnel employed in a plant which used dimethyl nitrosamine as a chemical intermediate in the manufacture of unsymmetrical dimethyl hydrazine, used as rocket fuel. Production was initiated in 1956, and discontinued in 1976.

In 1980, studies were begun on nitrosamine exposure in the manufacture of leather in cooperation with the Leather and Fur Workers' Union. Significant nitrosamine exposure occurs during leather tanning and the possibility of establishing a suitable cohort of long-term leather tannery workers is being explored. Records are being reviewed in New York, Newark, Massachusetts and other states.

During 1980-81, the potential for field study of additional methodological approaches to the problem of reproductive effects of environmental agents was investigated. Cesar Chavez's Farm Workers' Union was visited in Keene, California,

to explore the possibility of a prospective study of reproductive experience of farm workers, both male and female, exposed to pesticides and chemicals in their work.

Basic approaches to the collection of data relating to human reproductive experience are planned in connection with the fluorine field studies at the St. Regis Reservation. A number of laboratory studies have been reported, raising questions whether genetic or embryotoxic effects might occur with fluorine exposure. The reproductive experience of Mohawk Indians of child-bearing age resident in the St. Regis Reservation will be studied in comparison with the reproductive experience of Mohawk Indians who reside in the control area at Caughnawaga. The study design is based upon serial, prospective observation of the menstrual and reproductive experience of the populations concerned, including miscarriages, pregnancy interruptions, stillbirths, status of delivered infants, including birth weight and size and the presence or absence of congenital malformations, either in the immediate postpartum period or subsequently. Since the study design is based upon prospective surveillance, it will be possible to ascertain the relationship of these various findings with initial health status recorded during the clinical field surveys.

In 1968, data became available demonstrating an important multiple factor interaction between asbestos and cigarette smoking. These were recently extended in a group of asbestos factory workers, not employed in the construction industry.

This Laboratory continues to maintain close interest in problems associated with multiple factor interaction. These include not only those relating to cigarette smoking but also problems associated with immunosuppression following exposure to halogenated aromatic hydrocarbons and other xenobiotics and the effects of altered metabolism associated with enzyme induction.

Coal samples are being examined by rank, with fixed carbon and volatile content determined. Anthracite, bituminous and lignite samples have been examined by polarized light microscopy, x-ray diffraction, transmission electron microscopy and analytical electron microscopy. Increment heating and gravimetric determination of ash content (and its composition) have been determined. It is evident that the coal samples available appear to contain substantial quantities of quartz and kaolin. Electron microscopy also indicates that all specimens contain substantial numbers of particles smaller than 5μ. In some instances, 50% of the particles were smaller than 1μ in their greatest dimension. The specimen with the lowest ash content, bituminous coal, contained 73% "pure coal"; the anthracite specimen contained 51% "coal"; the lignite contained no "pure" coal. Thus, high ash content coals will produce dust in the respirable range, and the impure aggregates will often contain quartz.

These preliminary investigations suggest that coal specimens need be very carefully characterized before biological testing. Their mineralogical and chemical variability is extremely high and the particle population is such that individual analysis within cells may be required to interpret biological results.

University of California - Berkeley

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, the Ames group has developed some new tester strains which detect some of the oxidants caused by lipid peroxidation. They have also made some progress on a method of fusing mammalian microsomes to the membrane surface of Salmonella.

A second class of "false negatives" in the Salmonella test are natural carcinogens present in the human diet as glycosides. In order to detect these carcinogens, one needs a model for the metabolism of the bacteria in the human colon, as some glycosides are split by these bacteria to liberate mutagens. The Ames group has now developed such a model that works quite well for this whole class of compounds. They have made an enzyme preparation, which they call fecalase, by sonicating human feces (which is made up of bacteria to a large extent) and have shown that fecalase contains a wide variety of enzymes splitting sugars from glycosides. By adding fecalase to the Salmonella test, they have also shown that many different naturally occurring glycosides of mutagens (flavonoids, anthraquinones, cycasin, etc.) now show up as mutagens. Many beverages, including red wine (but not white wine), red grape juice, and tea, were shown to contain glycosides of the mutagen quercetin, which has recently been shown to be carcinogenic by others. Red wine, red grape juice and tea were mutagenic when fecalase was added, and red wine contained considerable direct mutagenic activity in the absence of fecalase due to the hydrolysis of the glycosides during fermentation.

The major RNase of human urine, a pancreatic-like RNase of unprecedentedly high molecular weight, has been purified and subjected to structural analysis. Glycosylation appears to account for its large size and its heterogeneity. The RNases of cerebrospinal fluid (CSF) have been shown to be glycoproteins related to the RNases of plasma; RNase species unique to CSF have altered carbohydrate side chains relative to counterpart species in plasma.

The plasma RNase activity of newly diagnosed lung cancer patients, widely reported to be elevated, has been shown to be unaffected by removal of the primary tumor. Thus, plasma RNase is not a tumor marker in lung cancer.

The levels and electrophoretic profiles of CSF RNase activity in normal individuals and in individuals with a variety of psychiatric and neurologic disorders have been examined and found to be indistinguishable.

A uridine substituted polyacrylamide has been synthesized and shown to be effective in affinity electrophoresis of RNase A.

In addition, the mitochondrial DNA sequence in over 70 human individuals representing all the major geographic groups of Homo sapiens have been compared by restriction methods. Surprisingly, the levels of variability among human individuals is not much higher than that for nuclear DNA. By contrast, in apes the levels of mitochondrial DNA sequence variability within species are far higher than the nuclear levels. These results may imply that an unusual event took place in human evolution. This event could have been the acquisition of an improved mechanism for protecting or repairing mitochondrial DNA.

With the DNA transfer and hybridization techniques developed by Southern, Dr. Wilson's lab prepared more detailed maps of the globin gene regions of five ape species and compared them to those of the human. These studies confirm that the α globin gene region has been highly unstable in primate evolution. The mechanism most likely to account for this rapid evolution seems to be intergenic recombination. Wilson and his collaborators propose that intron length, intergenic distance between duplicate genes, and differential regulatory elements between duplicates are affecting the probability of intergenic recombination.

In a related study, the nucleotide sequence of the globin cDNAs of four non-human primate species were compared to published sequences for human, rabbit, and mouse. It appears that point mutations have accumulated more slowly in the non-coding regions of the β genes of primates than is the case for rabbit or mouse lineages. Yet the primate β coding regions have evolved by point mutation at about the same rate as in other mammals. Attention is now focused on the question of why evolutionary change has been retarded in the noncoding regions of primate β globin genes.

Center at University of Rochester

The center is comprised of four major research programs, as follows:

1. Methylmercury in Human Populations. The rich data source provided by the extensive poisoning outbreak in Iraq during 1971-1972 is still being exploited, largely because a stable exposed population was identified, and because of methylmercury's enhanced threat to the developing organism. Children exposed perinatally are being followed to determine the extent of impaired development. Fish-eating populations and pertinent animal models are also under study.

In mothers, signs and symptoms of poisoning is typical of methylmercury poisoning in adults. Sensory, visual motor and cerebellar dysfunction dominated the clinical picture. The mothers were classified into three groups: 1) asymptomatic (no signs or symptoms); 2) symptoms only; 3) signs and symptoms. Mothers having only subjective complaints (symptoms only) would correspond to the "mild" category and those who had both signs and symptoms would correspond to the "moderate" to "very severe" category as proposed by Damluji for classifying Iraqi cases of methyl-mercury poisoning.

In infants, there was a combination of delay in motor development, language development, and toilet training, together with brisk deep tendon reflexes and sometimes a bilaterally positive Babinski's reflex, indicating damage to the central nervous system.

In studies of fish-eating populations, a follow-up visit was made to two fishing villages - Mancora and Cancas. 190 villagers were examined clinically and 260 gave a medical history. Blood samples were obtained from 182. The blood MeHg concentrations ranged between 11 and 275 ng/ml, distributed as follows (ng/ml): in 72 persons, 11-50; in 72, 51-100; in 33, 101-159; in 10, 151-200; and in 5, over 200. The mean concentration was 82 ng/ml. In Morropon the concentrations were much lower, ranging between 3.3 and 25.1, ng/ml, with a mean of 9.9 ng/ml.

2. Behavioral Toxicology. In comparative studies of the toxicity of cadmium, methylmercury and lead, several similarities and differences were found.

Two bottle preference tests in rats were conducted to determine the aversion function more precisely for Cd. Tests were conducted with 0 versus 10, 30 or 90 ppm and 0 versus 1, 3 or 10 ppm. Dramatic decrements in fluid consumption were observed in all rats at 10, 30 and 90 ppm. At the lower concentrations some rats rejected solutions as low as 1 ppm. Control rats drank much more fluid at 0.15 percent and 0.30 percent saccharin and progressively less at the three higher concentrations. Cadmium-treated rats drank slightly more fluid at the three lowest saccharin concentrations and progressively less at the two highest values. When the difference scores for the two groups for distilled water versus the various saccharin concentrations were compared, significant differences were found at all concentrations except 1.0 percent. No concentration of saccharin reduced the aversive properties of the 150 ppm cadmium solution.

Toluene is being studied as a representative solvent in an effort to explore the generality of the stimulus control variable in determining the effects of chemicals. Rats were exposed to toluene concentrations up to 3000 ppm for four hours immediately before their operant behavior was tested. Toluene reduced the accuracy of both types of performance in a concentration-related fashion. Although changes in behavior occasionally appeared at lower concentrations, clear differential effects occurred at 1780 ppm, with the behavior under external stimulus control showing more resistance to the acute behavioral toxicity of toluene. A variation of this procedure described below will be used to investigate tolerance to toluene.

Tests were completed with rats trained to press a lever for food. On a fixed interval 5-minute schedule of food presentation, ozone decreased response rates. Ozone concentrations ranged from 0.1 to 1.0 ppm, and exposures were conducted weekly for 6-hour periods. Concentrations above 0.2 ppm seemed effective.

Subsequently rats were allowed access to a running wheel for six hours daily. Such voluntary activity was sensitive to as little as 0.12 ppm of ozone when successive exposures were separated by four days or more. Analysis of the pattern of running indicates that it occurred in bursts, and that intervals between bursts were increased in a concentration-dependent manner.

3. Inhalation Toxicology. The time course of the exhalation of mercury vapor was studied after the exposure of acatalasemic mice to metallic mercury vapor. The accelerated exhalation of mercury was observed over periods of several hours compared with the corresponding control in catalase activity. There was increased exhalation of mercury even after dosing with mercuric chloride. These observations indicate that mercury is a dynamic equilibrium between metallic and mercuric mercury in the body.

The importance of the pulmonary lymphatic system in the removal of lung deposits was studied by measuring the accumulation of heavy metals in lung lymph nodes. Using dogs as the experimental model, it was found that intrabronchially administered cadmium is cleared via lymphatic pathways and that repeated exposure leads to accelerated clearance.

4. Developmental, Reproductive and Genetic Toxicology. Currently available information does not provide an adequate basis for constructing a detailed model of methylmercury metabolism and excretion. Reliable data are not available on sites and mechanisms of biotransformation of ingested methylmercury even in the adult animal. Recent investigations have revealed major changes in methylmercury

elimination in the mouse at different developmental stages and in adult animals fed different diets. It is apparent that a number of factors, including developmental changes and dietary influences, may affect metabolism, distribution and excretion of mercury compounds, especially methylmercury.

Age-dependent changes in rates of demethylation of methylmercury and in form of mercury excreted in feces have been observed. Results to date provide strong evidence that the developmental change in absolute rates of fecal excretion of mercury is mainly the result of an abrupt developmental change in rate of demethylation. These observations may be of direct relevance in evaluating hazards of methylmercury exposure in human populations. Studies are planned to determine whether similar developmental changes in excretion occur in the exposed human infant. If the human newborn and infant also excrete methylmercury very slowly, their risk from methylmercury accumulated in utero, or by suckling or other routes postnatally, may be greater than that of the adult.

It was found that diet strongly influences methylmercury excretion. Adult animals fed a milk diet excreted mercury much more slowly than animals fed the usual pellet diet. A defined high protein, low fat, low residue diet, markedly increased fecal mercury excretion after exposure to methylmercury. During the past year these studies were extended by comparing mercury excretion of animals exposed to radiolabeled mercuric mercury vs. radiolabeled methylmercury. It was found that in general diet had more influence on excretion of methylmercury than on mercuric mercury.

Studies on sexual differences in susceptibility to mercury have revealed that adult male and female mouse kidneys concentrate mercury differently and that this difference is related to testosterone stimulation. Sex-correlated differences in metabolic processing of mercury compounds are now being studied in terms of mouse strain, stage of development, cellular localization, and hormonal stimulation.

Studies in the mouse, as well as of exposed human populations, indicate that elimination of mercury after exposure to methylmercury may be affected by stage of development and diet. Recently it was observed that lactating mothers in the Iraqi population exposed to dietary methylmercury during the 1971-1972 methylmercury poisoning epidemic had a mean blood clearance half-time of 42 days compared with non-lactating females who had a mean half-time of 75 days. The ability of methylmercury to cross the placenta and to be secreted in breast milk are important considerations for hazard evaluation to fetus and suckling infant. In Iraq, breast milk levels as high as 90 ng/ml have been observed; however, this form of elimination alone is not sufficient to account for the 40 percent reduction in clearance half-times observed in lactating females in Iraq. Possible factors which may influence excretion of mercury and lead compounds during pregnancy and lactation include increased dietary intake, changes in hormonal metabolism and changes in trace element metabolism (selenium) in the pregnant or lactating animal.

In order to measure low levels of metallothionein in biological fluids there was a need to develop a more specific and sensitive method than those presently available. Antibodies have been developed in rabbits against purified rat hepatic metallothioneins. A radioimmunoassay has been developed in which ^{125}I-labeled metallothionein is used as an antigen. The assay is specific for metallothionein and there is no cross-reaction with beta-2 microglobulin, retinol-binding protein or serum albumin. Sensitivity of the assay is in the nanogram range.

A study in rats has been carried out in which the animals were given subcutaneous injections of cadmium chloride. Using the radioimmunoassay, metallothionein was detected in plasma as early as two weeks after the first injection and in urine after eight weeks.

The radioimmunoassay was also used to measure metallothionein in human urine. Spot urine samples were obtained from cadmium smelter workers with ten or more years of exposure and from "itai-itai" patients. The protein was detected in all samples. An epidemiological study to investigate the significance of urinary metallothionein in assessing excessive cadmium exposure is under way.

Kresge Center for Environmental Health
Harvard University

The activities of this Center are highlighted in the following four programs:

1. Radiation and Experimental Carcinogenesis. Factors that influence carcinogenic response to respiratory carcinogens were studied using instillations of ^{210}Po and benzo(a)pyrene (BP). Tumor development was preceded by the appearance of hyperplastic regions of bronchiolization of alveoli. Labeling indices in the alveolar region of the Po-treated group was slightly increased compared to untreated controls. Labeling of terminal bronchiolar cells was highest in the Po+BP groups and was associated with inflammation. A single saline instillation also increased proliferation of bronchiolar cells. It appears that both saline instillations and instillation of subcarcinogenic doses of BP may potentiate carcinogenesis in the hamster lung by acting as non-specific stimuli to cell proliferation. Although hyperplasia of alveolar regions preceded tumor formation in the Po and Po+BP groups, not all hyperplastic lesions in these groups progress on to form tumors.

In the continuing studies on the mechanism of malignant transformation, research has focused on the influence of tumor promoters and protease inhibitors on the process.

It was shown previously that post-irradiation incubation with protease inhibitors can markedly suppress the induction of malignant transformation by x-rays in 10T½ cells. In order to gain an insight into the mechanisms for this effect, a series of experiments were initiated to identify, quantify, and modulate through inhibition studies proteases associated with 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated mammalian cells. The initial protease studied was plasminogen activator (PA), a serine protease which has received attention in recent years because of evidence correlating the appearance of this protease enzyme with the neoplastic state. The minimal levels of PA activity of parental mouse cells could be stimulated up to 6 fold after a 4-day continuous exposure to concentrations as low as 0.1 ng/ml TPA. Moderately high levels of PA activity were present in both transformed cell lines and were not further stimulated by TPA incubation.

Alkaline and neutral elution techniques have been used to examine and quantify damage induced in DNA by the decay of incorporated ^{3}H-thymidine and ^{125}I-iododeoxyuridine in BALB/3T3 cells. This work was performed in parallel with studies on the in vitro transformation of 3T3 cells by these same radionuclides. A method has been employed in which cells are stored frozen at -90°C

to allow time for the accumulation of DNA damage after radionuclide incorporation. After various periods of time, the cells are thawed and those cells that attach to a Petri dish upon plating are assayed for DNA repair. This procedure eliminates cells killed by the freeze-thaw cycle. Thus far it was demonstrated that both single- and double-strand breaks result from the decay of these radionuclides in DNA.

2. Pulmonary Biology and Inhalation Toxicology. A short term assay system to measure the response of the lung to dusts was developed. The responses have been shown to be dose-related. Some of the indicators such as lactate dehydrogenase and PMN number seem to reflect the increased dust burden within the lung and are not very dust specific. Other indicators, such as albumin levels, β-N-acetylglucosaminidase levels, and particle endocytosis appear to reflect the toxicity of the dust as well. The system can distinguish between a very toxic dust, α-quartz, and a fairly inert one, aluminum oxide. However, it cannot as yet differentiate between talc and granite dust despite differences in disease risk for the two dusts. The response of the lung to a dust with respect to time is now being examined, since the chronic toxicity of a dust may be due to a prolongation of the short term response.

3. Biochemical Toxicology. Much of the work reported in this section has little to do with toxicology or environmental health. However, a few of the projects appear to have developing toxicological components, so that future reports may be more relevant to NIEHS.

4. Epidemiologic Studies. A mortality study was conducted to determine whether employment in the shoe and leather industries in Massachusetts was associated with excess deaths due to cancer. There was no significantly elevated mortality due to cancer among the shoe and leather workers on total or in any five year age stratum. However, a slight numerical excess of cancer deaths was seen among male shoe workers.

Analysis of cancer deaths by site demonstrated a statistically significant excess of digestive tract cancer among male shoe workers. No individual site of digestive tract cancer was present in statistically significant excess, although a numerical excess of stomach cancer was present. Female shoe workers had a statistically significant excess of bladder cancer. A numerical excess of digestive tract cancer was seen among male leather workers and a statistically significant excess of stomach cancer was present in this group. There were no other statistically significant excess deaths due to cancer.

The case referent analysis demonstrated a statistically significant excess of lung cancer among leather tanners. No other cancer was present in significant excess in any of the four employment groups. The results of this study support previous reports of bladder cancer hazards in the leather products industry. This study also suggests an association between work in the shoe industry and cancer of the digestive tract and between work in the leather industry and cancer of the stomach among males. These associations have not been reported previously.

In follow-up studies of workers who in 1980 had bladder dysfunction from exposure to Dimethylaminopropionitrile (DMAPN), the current status of symptoms and signs of this disorder was determined. These workers had been identified in follow-up interviews three months after the original study which showed that 67% of employees

at a polyurethane foam manufacturing plant had developed various symptoms of urologic dysfunction following exposure to DMAPN. Since these workers had persistent symptoms three months after removal from exposure to this chemical, concern existed that they were at increased risk for the development of permanent damage. The prevalence of urologic symptoms (urinary hesitancy, need to strain to initiate urination, and subjective feeling of bladder retention) was lower in 1980 than in 1978. Nevertheless, a considerable proportion still had symptoms. The severity of these symptoms was considerably less than that experienced during the acute phase of their illness. In contrast, prevalence of sexual difficulties (loss of libido, or impaired sexual function) increased in the group between 1978 and 1980.

Neurologic examination was performed only on those individuals with persistent symptoms of urologic or neurologic disorders. This examination showed only one individual out of ten with characteristic manifestations of sensory-motor neuropathy. Nerve conduction studies revealed virtually normal results in peroneal nerve testing but some abnormalities in testing of the sural nerve.

The Six-City Study on the health effects of SO_2 and particulates has completed six years of data collection. This means two rounds of surveys on each of the adult populations have been completed. The children have been surveyed each year. Children in all the schools in the town of Steubenville, Ohio, have had their pulmonary function measured each spring since 1976. In addition, an annual questionnaire is completed by their parents regarding their respiratory disease history and their exposure to cigarette smoke and other sources of pollution in the home.

It has been demonstrated that a temporary effect of peaks of air pollution on lung function of school children exists. The impairment of lung function, though relatively small in magnitude, seems to develop more slowly, and the recovery takes more time than had been previously believed.

These conclusions have been further strengthened when the study was repeated after one year, in November-December 1979, in the same schools. An additional feature in this study was inclusion of a sham alert. No significant change in FVC, relative to baseline values, was observed after the sham alert, in contrast to the sharp decline in function values when a real alert occurred within one week.

Additional analyses are needed to ensure that this observation is not an artifact of the study. It also may be that a few children are reacting strongly and, therefore, driving the entire observations. This needs to be ruled in or out.

Center for Health Effects of Fossil Fuels Utilization
Massachusetts Institute of Technology

Significant progress has been achieved among the various projects in the three component units: engineering, analytical chemistry and experimental biology. In the engineering unit, studies in certain projects were somewhat limited because of necessary modifications to reactor design and construction after initial experimental runs.

The effort in studies with the well-stirred reactor has been primarily devoted to development and construction of the combustion and sampling system. A series of material failures have forced continued development of reactor design and operation. Comparison of the PAH composition, obtained with toluene fuel, with results from the laminar flat flame burner show that while many of the same compounds are formed, production of the higher molecular weight and toxic compounds such as fluoranthene and the benzopyrenes increase after the combustion products leave the well-stirred reactor zone and that the proportions of these compounds compared to compounds such as napthalene and fluorene is higher than was found to be the case for the laminar flame.

Laminar flat flame combustion of benzene and toluene has resulted in new data on partitioning of polycyclic aromatic hydrocarbons (PAH), on soot and PAH yields as a function of fuel equivalence ratio, or mutagenicity of these materials, and on particle size distributions in toluene-derived soot. To achieve these results, a suitable burner system, sampling system, and sampling procedures were developed.

The combustion of pulverized coal particles in a laboratory furnace, under conditions of interest in industrial combustors, was found to produce quantities of soot and polycyclic aromatic hydrocarbons (PAH) which decrease very strongly with an increase in the concentration of oxygen in the gas surrounding the particles. Furthermore, Pittsburgh #8 Bituminous was found to produce greater quantities of PAH than the Montana Lignite at all temperature levels studied. PAH production peaked at 1250^{o}K for both the coals studied. This could possibly be due to secondary pyrolysis occurring at higher temperatures. From the above results, it seems clear that the effect of oxygen on the production of PAH needs to be studied in greater detail and that the initial steps of coal combustion, including particle heating and devolatilization, are probably critical with respect to soot and PAH formation.

Preliminary experiments using Solvent Refined Coal (SRC-II) in the fuel oil studies also indicate that PAH production was greater at 1273^{o}K as compared to 1173^{o}K. Also, PAH production in an atmosphere containing oxygen was substantially lower than that in an inert atmosphere, at 1273^{o}K. These initial runs also indicate that PAH compounds larger than 4-ring in size are being synthesized.

The main research effort in the Engineering Unit was directed towards the development of techniques and procedures for the collection of representative PAH samples both at points within turbulent flames and from the flue-gas, and also from the freeboard of fluidized coal combustors. The sampling problem is due to several factors. (a) The samples consist of several phases--partially burned combustible solids, fly ash, soot, liquid fuel droplets, gas and condensible vapors and these phases are difficult to separate. (b) Each element of the sample has to be quenched efficiently and it has to be ensured that condensation occurs only in the desired place in the sampling train.

In addition to the development of PAH sampling systems, a considerable effort has been expended for the development of non-intrusive, laser diagnostic systems which will be used to measure the spatial distributions of velocity, particle concentrations and particle size throughout the flame and combustion chamber.

In order to evaluate the composition of the mixtures of organic compounds produced by combustion of various fuels, they have to be analyzed by the combination of a powerful separation technique and a specific identification system. The most

generally applicable of such combinations is a capillary gas chromatograph with a mass spectrometer. This approach is being used to identify as many components as possible of a selected number of combustion product fractions to allow correlation of this selected set of data with the capillary gas chromatograms obtained under practically identical conditions from closely related experiments. It is clear that under certain conditions oxygen- and nitrogen- containing compounds will be formed and from certain fuels sulfur-containing substances may also result. The mass spectrometer which is now being used for these analyses has sufficient resolving power to permit the determination of the elemental composition of each compound and this greatly aids in the identification of such hetero-atom-containing substances.

A new, more powerful gas chromatograph mass spectrometer unit is now being used for the analysis of polycyclic aromatic compounds generated in the combustion experiments described in the other projects. It is expected that this approach will lead to a more complete identification of a larger number of components of each mixture being analyzed.

Over 200 compounds have been assayed for mutagenic activity using forward mutation to 8-azaguanine resistance as a genetic marker in S.typhimurium strain TM677 since regular operation of a dedicated laboratory began. In addition, many crude extracts of soots and other environmental samples have been assayed using the methodology developed. Of the 84 polycyclic aromatic hydrocarbon compounds assayed, 44 were found to be active mutagens.

The centralization of bacterial mutation assay in one laboratory has been a significant organizational improvement. Not the least important is that a knowledge of the day-to-day variations for negative and positive controls now rests on over 1500 results from untreated control cultures and many hundreds of assays for positive control compounds such as benzo(a)pyrene, 6-aminochrysene and nitroquinoline oxide.

In the course of this work a new mechanism of biological damage, induced by the model aza-heterocyclic pollutant acridine in the presence of sunlight, was found. Studies revealed that though the acridines are bound by the DNA of the bacteriophage, the lethal damage on absorption of a photon is to proteins closely associated with the DNA. This damage to proteins is dependent upon the acridine being bound to the DNA; the proteins are unaffected by exposure to acridine and sunlight in the absence of DNA. The lethal damage does not involve cross-linking phenomena, as is found with UV light, but probably proceeds via an active singlet oxygen species attacking amino acids in the proteins.

Oregon State University

The research in this Center deals with toxicology, metabolism, detection, quantitation and chemodynamics of such materials as the chlorinated cyclodienes (e.g. dieldrin), methylmercury, DDT, PCBs, chlorinated phenoxy acids, halogenated alkanes, plant alkaloids and cadmium. It also deals with problems of bioaccumulation through food chains and other environmental media, metabolism, carcinogenicity, and transport and behavior of chemicals in the environment. New analytical techniques were developed in support of these programs and attempts were made to develop a hazard assessment with respect to human exposure.

Pyrrolizidine alkaloids and their derivatives were used as alkylating agents to study induction of viral gene expression. Various alkaloid structures were examined to determine if structural changes alter the ability of the chemical to induce virus synthesis. Virus synthesis was measured by infectivity measurements of culture fluids from induced cells with a susceptible cell line (line 15B C/C chick embryo fibroblasts) and by synthesis of viral protein using a fluorescent antibody staining reaction.

Strong evidence was obtained that the alkaloids will induce the synthesis of RNA tumor virus in chick embryo fibroblasts. Modification of the basic structure of the alkaloids in the region of the carbons 7 and 9 will alter the ability of the compound to induce gene expression. Preparation of a ^{3}HcDNA probe complementary to unique base sequences in the AMV genome provides a hybridization probe for the synthesis of mRNA corresponding to what is believed to be the transforming component of the viral genome. This probe will hybridize to AMV viral RNA but no other viral RNA. The AMV specified sequence of this virus that causes leukemia in chicks is also present in normal cell DNA in at least one copy per haploid cell genome.

Gravid female rainbow trout were exposed to dietary Aroclor 1254 (PCB) for two months prior to spawning to deposit PCB in the lipid of the eggs. Levels of PCB in the eggs were measured, effects of PCB on liver tumor incidence initiated by embryo exposure to aflatoxin B_1 (AFB_1) were noted, and hepatic mixed function oxidase (MFO) enzyme activities were determined.

PCBs were present in the lipids of the eggs at significantly higher levels than the background levels present in control eggs. Liver carcinoma incidence was significantly higher in the offspring from the PCB-exposed female at both 9 months and 12 months, indicating that pre-induction of the liver by PCB enhances aflatoxin-initiated carcinogenesis. Mixed function oxidase determinations also revealed trends that suggest induction of the MFO system by PCB. Cytochrome P-450 content in the PCB fry livers was 174% of the control values, while the *in vitro* conversion of AFB_1 to aflatoxicol (AFL) was 264% of control. These results call attention to the possible role of PCB in enhancing carcinogenesis.

Mice were exposed to lead, cadmium, methylmercury (MeHg), or combinations of MeHg with Pb or Cd for 10 weeks. Mice given any one of the metals had significantly reduced numbers of antibody producing cells as compared to controls. However, the combined effects on mice of exposure to Pb or Cd and MeHg were not quantitatively different from mice which received only one of the metals. Residues of Hg were decreased in tissues of mice exposed to Pb or Cd while Pb or Cd residues in renal tissue were increased. It appears as though some interaction occurs between Pb or Cd and MeHg in regard to antibody synthesis and tissue residues of these metals. No significant interaction was evident between virus-induced mortality in mice and exposure to combinations of metals.

Aroclor 1254 (100 ppm) was fed to rainbow trout for 15 weeks to determine the effects on hepatic microsomal enzyme induction. Fish were also fed combined PCBs and cyclopropene fatty acids (CPFA) tc determine the antagonistic effects on mixed function oxidase (MFO) induction.

Dietary PCBs markedly induced the microsomal activities of 7-ethoxy-resorufin O-deethylase, 7-ethoxycoumarin O-deethylase, and benzo(a)pyrene monooxygenase. 7-ethoxyresorufin O-deethylase activity continued to increase to 77-fold higher

than control at week 15. 7-ethoxycoumarin O-deethylase and benzo(a)pyrene monooxygenase activities increased to 7.1-fold and 48-fold over the control at week 9 and then slightly decreased to 6.8-fold and 45-fold over the control at week 15, respectively. Cytochrome P-450 values remained approximately 2-fold above controls from week 5 through week 15.

7-ethoxyresorufin O-deethylase, 7-ethoxycoumarin O-deethylase, and benzo(a)pyrene monooxygenase activities in the combind diet PCB and CPFA-fed trout were significantly higher than in controls and CPFA-fed fish, and significantly lower than in PCB-fed fish. There was no significant difference in cytochrome P-450 levels after week 5.

This is the first time a dietary level of PCBs has been shown to induce the MFO system in PCB-fed rainbow trout.

Aflatoxin Q_1 (AFQ_1), the major microsomal biotransformation product of aflatoxin B_1 (AFB_1) formed in vitro by monkey and human liver preparations, was fed to rainbow trout in a semipurified diet at levels of 20 ppb for 1 year and 100 ppb for 10 months. As a test for carcinogenicity in hatched fish, it was also used at 1 ppm in a water solution and exposed for 1 hour to fertile trout eggs. The 20-ppb dietary exposure and 1-ppm egg exposure failed to elicit a carcinogenic response; however, the 100-ppb dietary exposure produced a 10.6% incidence of hepatocellular carcinomas at the end of 1 year. Administration of 50 ppm methyl sterculate, a cyclopropenoid fatty acid, in combination with 100 ppb AFQ_1 resulted in a synergistic tumor response similar to that previously noted with administration of AFB_1 and aflatoxin M_1. This comparison of carcinogenic potencies was very similar to the comparison of the relative mutagenicities of the two compounds in the Ames bacterial mutagen assay system.

Center in Environmental Toxicology
Vanderbilt University

Included as current research topics are toxic fungus and higher plant metabolites, pesticides, mutagenic, carcinogenic, teratogenic agents and toxic metals.

Investigation into the toxicity of moniliformin has continued. Moniliformin is a highly toxic fungal metabolite produced by Fusarium moniliforme, a common contaminant of grain. The compound is a vinylogous α-ketoacid and interferes with mitochondrial pyruvate and α-ketoglutarate oxidations. The inhibition of pyruvate dehydrogenase and transketolase from rat liver and brain has been studied.

The enzymatic monooxygenation of halogen atoms is a third area investigated in the past year. Cytochrome P-450 was found to catalyze the transfer of oxygen from iodosobenzene to iodobenzene. This was determined by incubating ^{125}I-iodobenzene with purified P-450 and unlabeled iodosebenzene and determining the amount of ^{125}I-isodosobenzene formed by HPLC. The oxygen transfer requires P-450 and is not effected by other heme proteins such as horseradish peroxidase or catalase.

Methods have been developed to purify human liver cytochrome P-450, NADPH-cytochrome P-450 reductase and epoxide hydrolase to apparent homogeneity. These methods are now being used routinely to prepare such enzyme fractions from a single source of microsomes. Antibodies have been raised in rabbits against human liver

cytochrome P-450 and epoxide hydrolase and used to examine the variation of these enzymes in human populations. Antibodies raised against rat liver NADPH-cytochrome P-450 reductase have also been used to study the interspecies variation of that antigen. Simpler and better methods have also been developed for the purification of rat liver enzymes, and now cytochrome P-450, epoxide hydrolase, and NADPH-cytochrome P-450 reductase can be isolated in overall yields as high as 20, 25, and 60%, respectively, from a single preparation of microsomes. Immunological inhibition of the regio- and steroselective metabolism of warfarin metabolism has also been used to demonstrate that different forms of rat liver cytochrome P-450 bind to a common NADPH-cytochrome P-450 reductase with different degrees of functional affinity under conditions which mimic the microsomal membrane. An alcohol dehydrogenase-coupled spectrophometric assay has been developed for the assay of epoxide hydrolase activity towards substrates lacking chromophores or radioactive labels. The role of epoxide hydrolase in blocking the binding of benzo(a)pyrene dihydrodiols to DNA in various reconstituted and cellular systems has been studied. Work on the immunohistochemical localization of cytochromes P-450 and epoxide hydrolase in rat liver tissues has continued and recent studies have been carried out in skin and pancreas as well as liver. Studies on the inhibition of irreversible binding of metabolites of vinyl chloride and vinyl bromide indicate that DNA is a preferred target for 2-haloethylene oxides and protein is the preferred target for 2-haloacetaldehydes, the rearrangement products of the epoxides. Other studies suggest that large amounts of reactive metabolites leave the hepatocytes before binding. This finding has relevance with the nature of vinyl halide-produced hemeangiosarcomas.

Investigations of chemical-induced placental toxicity has continued. Isolated apical microvillous surface membrane vesicles from human placental syncytiotrophoblast were investigated for chemical perturbations of α-aminoisobutyric acid (AIB) facilitated diffusion. The d and l stereoisomers of propranolol were equipotent in inhibiting AIB transport, indicating independence of AIB transport from the β-adrenergic blocking activity of l-propranolol. Quinidine, an antiarrythmic with local anesthetic effects, caused inhibition similar to propranolol. However, the membrane active compounds cocaine and lidocaine exhibited little, if any, inhibition of AIB transport into the vesicles. Chloroform, a teratogen, caused inhibition and lowered equilibrium levels of AIB in the vesicles. The apical microvillous membrane preparation is being used to further characterize the effect of chemical agents on transplacental transmembrane transport.

An experimental screening was completed of the relative efficacy of 29 compounds as antidotes for acute mercuric chloride intoxication. The purpose of this was to develop data for the formulation of structural requirements which compounds must satisfy if they are to be good antidotes for acute mercuric chloride intoxication. The most effective antidotes were N-acetyl-D,L-penicillamine, 2,3-dimercaptopropane-1-sulfonate and D-penicillamine. Each of these combines a high stability constant for the mercury(II) complex, a high degree of water solubility, and a relatively modest rate of metabolic transformation.

An analogous study was conducted on antidotes for nickel(II) involving 27 different chelate structures and 13 compounds were found which are very effective antidotes for acute nickel(II) intoxication. Here the prime requirement seems to be the formation of a water soluble nickel complex of low inherent toxicity, a feature which was more important than the particular structural features of the chelating agent.

A study of antidotes useful in acute and chronic copper intoxication has also been carried on and the initial stage of this has been completed. 2,3-Dimercaptopropane-1-sulfonate was found to be significantly superior as an antidote for copper(II) intoxication to any of the other compounds which have been investigated for this purpose. The level of copper(II) loading used in those studies was very high. As a consequence, the general behavior of compounds more modestly effective as antidotes was not clearly differentiated. Chelating agents based on N or S atoms, or mixtures of these, seemed to be much more effective antidotes than those based on O atoms.

α-Naphthylthiourea (ANTU) is metabolized by rat liver and lung microsomes to α-naphthylurea (ANU) and atomic sulfur. A portion of the atomic sulfur formed in this reaction covalently binds to macromolecules of the liver and lung microsomes. Approximately half the atomic sulfur bound to the liver and lung microsomes appears to have reacted with cysteine side chains of the microsomal proteins to form a hydrodisulfide. The loss of cytochrome P-450 and monooxygenase activity seen on incubation of liver microsomes with ANTU is likely the result of the covalent binding of atomic sulfur to cytochrome P-450. The available evidence suggests that the pulmonary toxicity of ANTU results, at least in part, from the covalent binding of a cytochrome P-450 monooxygenase catalyzed metabolite of ANTU to pulmonary macromolecules. This metabolite is most likely atomic sulfur or alternatively, one containing the carbonyl carbon of ANTU. However, it is possible that the binding of both metabolites may be responsible for the lung toxicity.

The effects of total parenteral nutrition (TPN) upon the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats was studied. At doses of 50 or 100 μg/kg TPN-fed, TCDD-treated rats demonstrated a weight gain similar to that of TPN-fed controls, but died at Days 13-17 following treatment. Gross examination of moribund animals revealed icterus, thymic atrophy, increased adipose tissue depots, and enlarged livers. The liver weights ranged from two to three times those from TPN-fed control animals. Histologically the livers were severely necrotic. Most cells which were not neucrotic were markedly swollen and disorganized. Extensive vacuolization of hepatocytes and cystic areas containing cell debris were also prominent features. Whereas glycogen stores were depleted, the total content of water, lipid, protein, RNA, and DNA in the livers was increased. Alterations in cytochrome P-450-associated monooxygenase activities were also observed. Statistically significant increases in serum iron, bilirubin, alkaline phosphatase, serum glutamic-oxaloacetic transminase and cholesterol were found in the TPN-fed, TCDD-treated animals.

Serum protein, glucose, and triglycerides were significantly decreased except in a few moribund animals in which hyperglycemia was observed. The results in the TPN-fed, TCDD-treated rats were compared with TCDD-treated rats fed a chow diet ad libitum. At the same dose of TCDD, the liver damage in the TPN-fed, TCDD-treated rats was histologically more severe.

The tissue distribution of ^{14}C-labeled TCDD in adult male guinea pigs was studied up to 15 days following its ip injection (2.0 μg/kg). On Day 1, the highest levels of radioactivity were located in the adipose tissue, adrenals, liver, spleen, intestine, and skin. All other tissues examined contained less than 0.3%/g tissue. By Day 15, the level of radioactivity in the liver increased to nearly three times its initial value. An increase in radioactivity was also noted in the adrenals, kidneys, and lungs. These increases appeared to be due to the mobilization of fat stores and the subsequent redistribution of radioactivity contained in these stores

to other organs. Following a single intraperitoneal dose of 0.5 μg (^{3}H)TCDD/kg the excretion of ^{3}H in the urine and feces appeared to be linear up to 23 days. Assuming the excretion of radioactivity would continue in a linear manner, the time for excretion of half the administered dose by way of the urine and feces was calculated to be 30.2 ± 5.8 days. The effect of TCDD (1.0 μg/kg) upon various clinical chemical parameters was determined periodically up to 14 days and compared to pair-fed controls. Statistically significant increases in plasma albumin, total protein, iron, urea nitrogen, cholesterol, and triglycerides were observed in TCDD-treated pigs.

AIR POLLUTANTS AND RESPIRATORY DISEASE

Exposure of mice to submicron-sized sulfate particles, nitrogen dioxide and ozone is being carried out to determine the effects with respect to challenge by minimally virulent S. aureus and virulent group C. Streptococcus. Sulfate particle exposure did not interfere with the ability of the lung to kill the S. aureus, however the capacity to kill the Streptococcus was impaired and was associated with earlier, but not increased, overall mortality. When nitrogen dioxide at 1.0 ppm was an added insult to the mice, enhanced susceptibility to Streptococcal infection was noted in a manner suggesting that each pollutant acted independently.

Angiotension converting enzyme (ACE) in serum is being used to evaluate acute lung damage in rodents, sheep, dogs and in human patients suffering with a variety of well-defined acute lung disease. A variety of lung insults are employed, including exposure to thiourea, paraquat, bacterial infection and cardiogenic and non-cardiogenic pulmonary edema. Studies to date indicate that there is a higher degree of correlation between acute lung injury and elevated ACE in the animal models employed than in studies on man. These findings may relate to the ready availability of sampling in animals as opposed to man, at times when ACE levels are elevated.

Many industrial chemicals are known to cause pulmonary hypersensitivity in exposed individuals. The immune response may occur immediately after exposure or be delayed and occur only several hours after exposure. Using the guinea pig animal model, a plethysmograph is being employed to study respiratory rate and tidal volume in sensitized and control animals. The use of such a system should allow for complete assessment of the pulmonary sensitizing properties of industrial chemicals.

Generation of singlet oxygen was accomplished by three methods--laser excitation, heterogeneous photosensitization and direct absorption of irradiation in the study of the role of singlet oxygen in lung pathogenesis. It was found that singlet oxygen was formed in the generation system when the sensitizer was removed and the first report that direct absorption of radiation by ground state oxygen at atmospheric pressure is being prepared. Experiments performed using heterogeneous photosensitization were conducted under conditions that closely approximate the environmental situation. Studies are continuing to determine the role of singlet oxygen in producing deleterious effects on lung surfactant and tissues and to investigate possible reactions with olefins in the atmosphere to form toxic hydroperoxides and with polycyclic aromatic hydrocarbons.

In vivo and in vitro effects of asbestos and related fibers in mouse peritoneal macrophages are being followed by evaluating the macrophage products, plasminogen activator, collagenase and prostaglandin. Macrophages exposed to IARC chrysolite B, thioglycolate medium and starch elicit high levels of plasminogen activator but low levels of prostaglandin E. A series of asbestos fibers including IARC chrysolite A, IARC crocidolite, IARC amosite and anthophilite were shown to induce exudate when injected intraperitoneally in mice. The exudate macrophages are also active producers of plasminogen activator. These findings

may be important in understanding the role of the inflammatory processes in carcinogenesis and tumor promotion.

Regional deposition of inhaled particles in man have shown that deposition in the trachea as well as in bronchial generations 1 to 5 were greater under cyclic inspiratory flow conditions than under constant flows. The differences observed were directly related to flow and inversely related to size, and were considered to be due to some factor affecting deposition which differs between constant and cyclic flow, such as the degree of turbulence or localized velocity profiles. Regional particle deposition studies were performed on 17 healthy, nonsmoking volunteers exposed to one of two different sized aerosols. Based on regional deposition fractions, bronchial deposition sizes were determined to be 1.24 and 1.75 for the two particles used.

Studies on lung carcinogen metabolism after exposure to air and 0.8 ppm x 4 hrs of ozone in rats which were sacrificed 30 min or 48 hrs after exposure showed that the rate of NADPH-dependent metabolic activation of ^{3}H 4-ipomeanol to intermediates which combine with GSH to form two GSH adducts was increased substantially in lung microsomes from both ozone-exposed groups when compared to controls. No change in the relative ratio of conjugates formed was observed. In contrast, lung microsomal metabolism of biphenyl to 4-hydroxybiphenyl was decreased to 70% of air-exposed control in animals sacrificed 30 min after exposure, but increased slightly in animals sacrificed 48 hours after exposure.

The pulmonary functional response to sulfur oxide components of air pollution in adolescent asthmatics has shown that the subjects are affected by inhalation of 0.5 ppm of SO_2 and that the pulmonary responses measured are dose-related, in that the response to 0.5 ppm is approximately one-half the magnitude of the response to 1.0 ppm of SO_2. Functional changes were seen in tests which are thought to reflect both changes in the large (R_T,FEV_1) and small (V_{max50} and V_{max75}) airways. Therefore the data suggest effect (and deposition) of SO_2 throughout the tracheobronchial system and it is to be expected that the results will be useful in determining public health standards for SO_2 exposure at the local and federal government levels.

Mathematical and computational models for aerosol deposition in the human respiratory system have shown that inhaled aerosols will deposit along the air passages between the point of entry at lips or nares and the larynx. The amount of aerosols entering the lung is therefore the fraction of aerosols that penetrates these passages. The major mechanism of deposition in this part of the respiratory system is by impaction. However, the details of particle mechanics is very complex, mainly due to the complexity of geometry. It was also found that, at the same lung volume, deposition is different for different lung models, but these differences are relatively small in total deposition and they become more profound for regional deposition. At generation-by-generation level, deposition profiles for different lung models are drastically different. Because different lung models differ mainly in their airway dimensions and the number of structures, the deposition results suggest the possibility of using aerosol measurements for the verification of a lung model. Using the deposition model, preliminary calculations have been made for extrafine particles. The results show that deposition varies with a parameter DT regardless of particle size, where D is the Brownian diffusion coefficient and T is the breathing period. The effects of tidal volume and flowrate are small. Theoretical estimates of the tracheobronchial deposition for charged particles in 1-10 μm size range

show significant enhancement in deposition. For example, inhaled particles having a mass medium aerodynamic diameter of 2 μm and 200 elementary charges per particle will result in T-B deposition of 39% as compared to 3% for uncharged particles of the same size during inhalation.

The effects of cigarette smoke and various other exogenous factors on pulmonary metabolism are being studied. Single exposure of rats to smoke during one hour increases the conversion of angiotensin I to angiotensin II and decreases the inactivation of prostaglandin E_2 in the isolated perfused lungs. When rats were exposed to nicotine for 1 or 10^2 days (1 mg/kg s.c. once daily) instead of cigarette smoke the conversion of angiotensin I and the inactivation of prostaglandin E_2 remained unaltered in the isolated perfused lungs. This suggests that the alterations caused by cigarette smoke would be due to other components of cigarettes rather than nicotine. When cigarette smoke was in contact with isolated superfused hamster stomach strip or rat colon, the contractile responses of these tissues to prostaglandin E_2 and angiotensin II respectively, were strongly inhibited. An inhibitory factor could be transported to the tissues also in the effluent from isolated rat or hamster lungs ventilated with cigarette smoke. Blood was as effective carrier of the inhibitory factor(s) as was Kreb's bicarbonate buffer. The inhibitors were not fully identified, as the inhibitory effects of nicotine and carbon monoxide could explain only part of the inhibition.

An <u>in vitro</u> model system using $P388D_1$ mouse macrophage tumor cells was developed in order to assess the causal relationship between specific metabolic and membrane changes and cell death caused by silica. While exposure to silica does not seem to disrupt the major structural components of $P388D_1$ cells, it remains possible that silica disrupts specific cell functions. Preliminary experiments to measure ATP levels in $P388D_1$ cells exposed to silica suggest that this is true. ATP levels rapidly decreased following exposure to 200 ug of silica. This decrease occurred before loss of viability. Thus, decreased levels of ATP may be causally related to the production of cell death and are not simply the result of cell death. Additional studies suggest that decreased ATP levels and a phagocytic stimulus are not sufficient to cause cell death. It is hypothesized that silica, in contrast to TiO_2, damages the cell membrane and that this membrane damage contributes significantly to cell death.

The pulmonary functional response to sulfur dioxide components of air pollution in adolescent asthmatics is being studied with the subjects both at rest and during moderate exercise. It has been determined that, at rest, adolescent asthmatic subjects are affected by inhalation of 0.5 ppm of sulfur dioxide and that the pulmonary responses measured are dose related in that the response to 0.5 ppm is approximately ½ the magnitude of the response to 1.0 ppm. From the lung functional changes observed, the data suggests effect and deposition of sulfur dioxide throughout the tracheobronchial system. The results of this research will be useful to determine public health standards for sulfur dioxide exposure at the local and federal government levels.

A study in the biochemical effects of ozone and nitrogen dioxide in the lung is in progress. The purpose of the study is to determine the biochemical mechanisms by which ozone and nitrogen dioxide produce their toxicity. Major efforts include: (1) a study of the toxicity of ozone due to the reactivity of malonaldehyde produced by ozone inhalation, (2) the formation of N-formylkynurenine by oxidation of tryptophan by ozone, (3) determination of pulmonary macrophages and

granulocytes, (4) determination if histamine released by activated complement is potentiated by ozone exposure, and (5) studying the combined effects of ozone and nitrogen dioxide. Low levels of ozone will be used in the hope of extrapolating detectable amounts of cross-linked materials to the ambient concentrations of ozone. Reaction of malonaldehyde with nuclear components will be investigated by studying the tumorgenic effects of long term exposure to ozone.

Quinoline and its derivatives occur in urban and indoor atmospheres, in engine exhausts, in industrial emissions, in consumer products, in tobacco smoke, and various fuels. A study to determine the prevalence of this compound is underway. A parallel study determining the mutagenicity of quinoline derivatives and their potential carcinogenicity will also be carried out. These studies deal with an important class of environmental pollutants which have received little previous study.

The effect of ozone on reproductive performance in mice is being examined. The influence of dietary vitamin E and synthetic antioxidants on these parameters will be examined and the concept of lipid peroxidation as a mechanism of toxicity of ozone will be investigated.

Studies are being carried out to elucidate the effect of nitrogen dioxide on the pulmonary system using various toxicity end-points such as the production of interferon by lung monocytes, and cell mediated immune responses in the lung. These studies are aimed at gaining information on the mechanisms by which oxidant pollutants mediate cellular dysfunctions in terms of specific and nonspecific immune responses.

The effect of ozone and nitrogen dioxide on the response of lung to bronchial constrictors is being studied using standard pulmonary parameters such as intrapleural pressures, tidal volumes, and rates of flow of gas to calculate compliance and flow resistance. Those response curves of air pollutants will be determined. Interactions of sulfur dioxide and nitrogen dioxide with carbon particles will also be investigated using this method, as will the effects of other particulates and gases such as formaldehyde.

The effects of air pollutants on cultured respiratory cells is under investigation. The cell types to be examined include tracheal epithelial cells, alveolar cells, and alveolar macrophages. Attempts will be made to determine the chemical events surrounding direct interaction of airborne pollutants with the specific cellular components.

Another study on sulfates and nitrates is to determine if the biological systems which maintain pulmonary sterility (mucociliary transport and alveolar macrophage function) are affected by inhalation of particles and gaseous pollutants. The model being used is an animal model using inhaled Staphylococcus aureus to determine bacterial clearance rates, bacterial activity rates, and the bacterial localization.

A new theory defining the initial biochemical response to ozone is under investigation. Specifically, determination of the effect of ozone on the cross-linking of red cell membrane proteins and the formation of mixed disulfides of red cell cytosol proteins is being used as a model for these events in type II lung cells. These studies should provide insight into the specific mechanisms of the susceptibility of proteins in general to ozone.

A study of the distribution and rate of inhaled sulfur dioxide or in just sulfites is being carried out. The metabolic capacity of several plasma and sulfur dioxides is being determined. The distribution and clearance rates of these products is also being determined.

PHYSICAL FACTORS

Preliminary studies of the electrical properties of cell surface using different techniques were carried out in order to address the problem of identifying and characterizing the mechanism by which extremely low frequency electromagnetic fields (ELF) affect living organisms. Using electrophoresis it was shown that the electrophoretic mobility of a cell is closely related to the zeta potential at the cell surface and that cells exposed to ELF have 10-20% lower mobility than control cells. Cell partitioning techniques showed that the partition coefficient for exposed cells differs by more than 20% from that of control cells. Using a luciferin-luciferase assay for ATP, exposed cells were shown to have an ATP level 15% lower than for control cells. The conclusion is reached that ELF affects energy metabolism either by affecting the transport of raw materials into the cell or by altering the control mechanisms present in the energy metabolism chain.

Attempts are being made to better understand the cutaneous responses to ultraviolet light in which a new method was developed for separating the lysis-inducing photoproducts of protriptyline. Using an action spectrum type study, the chlorpromazine molecule responsible for photo addition to DNA was identified--it was demonstrated that the action photoproduct from chlorpromazine was stable for hours, but reacted within minutes when DNA was added to the solution; and that covalent photoadducts of chlorpromazine with membrane products were shown to form during red cell lysis. In a related study the action spectrum for photosensitivity to musk ambrette in man and guinea pigs was established.

A new concept for the determination of the toxic hazard of environmental agents is being examined which suggests that the mutagenic/carcinogenic potentials of agents in the environment may be expressed in terms of exposure to ionizing radiation which produces the same effect (Radiation Equivalency). The assay developed utilizes antibody-dependent cell-mediated immunity (ADCC) in which it was found that rats which had only their hypoxic ileum and jejunum exposed to X-rays developed immunoglobulin IgG capable of inducing ADCC lysis, whether or not visible tumors were present. These findings indicated that if immunological responsiveness as delineated by ADCC plays a role in host tumor defense, then the deficiency in those animals developing lesions must lie in their circulating effector cells. Preliminary studies suggest that the cell mediated immunity and antibody-dependent cell-mediated immunity assays permit monitoring of perinatal (DMH and ^{131}I) and waste water contamination.

Qualitative and quantitative relations between characteristics of noise-induced (temporary) hearing loss are being studied. The effects of noise on physiological recordings of NVIII and auditory brainstem in a tuning curve paradigm have been completed and demonstrate that NVIII responses are clearly more affected by exposure to noise than one would predict from the audiogram or from brainstem responses. Audiometric correlates of noise-induced hearing loss provide insight into the mechanisms of hearing and the effects of noise on hearing. Procedures developed in the laboratory are being utilized for clinical use. Thus the SN_{10} response provides a unique assessment of the effects of noise, a unique method

to measure hearing in infants and is predicted to have wide clinical application in the near future.

Studies on the interaction of ultraviolet and sunscreen agents have shown that Escalol 506 and Escalol 507 (0.0025% final concentration) induce DNA damage and repair activity in mouse 10T½ cells as determined by alkaline sucrose gradient sedimentation profiles utilizing a bromodeoxyuridine (BrdUrd) photolysis procedure. PABA at final concentrations up to 0.1% did not appear to cause any DNA damage or repair activity. In studies in which both 254 nm light (UVC) and sunscreen treatments were utilized, Escalol 507 damage was enhanced by prior UVC light exposure. Survival experiments in 10T½ mouse fibroblasts showed that a protective effect does exist if the cells are irradiated after the sunscreen has been incubated with the cells. Having shown that the Escalol agents alone do cause DNA damage, their effects in human Xeroderma pigmentosum cells were determined. XP cells (complementation group A) did not appear to be differentially sensitive to killing by these compounds; i.e., the survival kinetics of XPA cells and normal human cells was similar. These results suggest that the damage induced by these agents is not acted upon by the excision repair pathway. The mutagenicity of these compounds has been studied in bacterial and mammalian cell systems. The compounds were negative in the Ames test strains TA100, TA98, TA1538, TA1537 and TA1535 (with and without S9) which include strains sensitive to both base pair and frameshift mutation. In a mammalian mutation test system (Chinese hamster V79 cells and 6-thioguanine resistance) the compounds were also inactive.

BIOLOGICAL AGENTS

Phylogenic relationships of occluded baculoviruses indicate that the baculoviruses have an ancient association with their insect hosts and the infectivity spectrum of these viruses has been confined to their respective order since divergence occurred. This conclusion has an obvious and important bearing on the use of these viruses for biological control of Insects. Thus techniques have been developed for identifying baculoviruses and for use in quality control of the viruses during their production for pest management.

Toxicological and chemical studies on sea nettle venom have demonstrated a neurotoxin which blocks peripheral nerve action potential and altered spontaneous transmitter release by non-specific depolarization. A kinin-like substance has been purified 20-fold which produces a cutaneous pain. Dermonecrosis was found to be induced by an aldaline protease, an acid protease or an endonulease. Mouse writhing is employed to measure cutaneous pain and cell culture, and agglutination techniques to measure dermonecrosis and lethality. Nettle cardiotoxin appears to exert an action on calcium transport and IgE antibodies cross-react with man-o-war venom in patients severely stung by the sea nettle, indicating that these individuals are "at risk" for systemic allergic reactions.

The structure and toxicity of Gymnodinium breve toxins is continuing and a degree of purity of the toxin T34 was established. Crystalline T34 was found to be composed of C, 61.63%; H8.15% with no nitrogen, sulfur, phosphorus and uncertain percentage of oxygen. T34 is an aldehyde and shown to be structurally-similar to a glycosylated steroid by nuclear magnetic resonance. Toxicity studies of the crystalline preparation indicate inhibition of the division of fertilized sea urchin eggs (8.9 mg/liter), toxicity to mosquito fish at 0.011 mg/liter, to swiss white mice at 0.2 mg/kg and for 3 different mammalian tissue culture cell lines at from 0.26 - 0.42 mg/liter. Contrasting T34 and T17 in nerve stimulated muscle

showed that T17 effects could be reversed, while T34 were irreversible.

Naturally occurring toxicants of animal and human foodstuffs are being studied including various aspects of the mechanism of action and structure-toxicity relationships of 3-substituted furans which occur either as stress metabolites or normal metabolites of certain plant foods. Experiments using perilla ketone and 4-ipomeanol have shown that repeated sub-lethal doses given to rats decrease the mixed function oxygenase activities of the liver microsomal fraction, although the cytochrome P-450 content was not altered. It was demonstrated that metyrapone, B-naphthoflavone and tetrahydrofuran inhibited microsomes from 4-ipomeanol and perilla ketone pre-treated animals. Differences in inhibition of induced microsomes may reflect the presence of only "control" levels of the various cytochromes P-450 in the microsomes.

Furanosesquinterpenes are produced by the sweet potato in response to stresses such as fungus infections. In the case of infection by certain fungi, potent lung toxins are produced by fungal catabolism of one or more of the stress metabolites. The isolation identification and study of the biosynthesis of the furanosesquinterpene metabolites and the investigation of the metabolic activation of the lung toxic agents in animals is under investigation. The complex chemical pathway leading to the ultimate toxic agent is under investigation to determine if the activating processes can be modified.

The chemical definition of the pain producing and lethal toxins in the venom of sea nettles is being undertaken. Steps toward chemical purification are being carried out and assays to detect the lethal and pain producing actions are being determined. An attempt is also being made to delineate the role of hypersensitivity to the toxins. Collagenases, proteases, and lectins have been determined as components of the venom but their roles are presently unknown. It is postulated that the proteases may be important in the production of pain, that the collagenases serve as a tool to allow the venom to disseminate within the tissues, and that the lectin alters nuclear structure or the integrity of the erythrocyte membranes.

The crystal structure of toxins is being studied to elucidate structure function relationships within a class of closely related proteins that display widely different toxic effects. The scorpion Centruroides sculpturatus Ewing is the source of the toxin. Scorpion venoms are complex mixtures that contain a number of small (M_r - 7000) basic proteins, which are responsible for the toxic effects of the venom. These toxic effects, which are of both cholinergic and adrenergic origin, are a consequence of the depolarization of receptive cells, e.g. neurons and muscle cells. Results of electrophysiological studies with those scorpion toxins that have been purified suggest that these proteins interact with sodium channels of excitable membranes to enhance the inward sodium current and to delay sodium current inactivation. This rather specific mode of action has stimulated a number of investigations in which scorpion toxins are used as probes to identify and characterize the sodium channel.

METALS

Mercury

The effect of selenium on glutathione metabolism and the mechanism of selenium protection against mercury toxicity studied in rats have shown that the Se

effect is exerted at the regulatory sites for the synthesis -glutamylcysteine synthetase and GSSG-reductase; and that the mechanism of Se protection against Hg toxicity involves increased synthesis by Se of the enzymes of GSH production. It appears that the observed increases in the activities of the synthetase and the reductase are either direct manifestations of Se effect on the turnover of -glutamylcysteine synthetase and glutathione disulfide reductase: or alternatively represent cellular responses to Se-mediated perturbations in the levels of reduced glutathione and glutathione disulfide. Studies on the effects of methylmercury on the erythrocyte membranes of newborn monkeys indicated the existence of major differences between the susceptibility of the newborn primate's blood cells to methylmercury in comparison to the adult animals. When erythrocytes of one-month-old newborn animals were exposed to methylmercury (50 M) a 3-4 fold increase in hemolysis in comparison to the control newborn blood cells was noted. Moreover, the red blood cell of one-month-old monkeys were, in general, more fragile than that of 4 month old monkeys. Interestingly, the red blood cells of lactating females showed a decreased sensitivity to methylmercury, a finding which may implicate the role of steroids in the methylmercury effect of the blood cells.

Studies on the chemical behavior of organomercury compounds have provided convincing evidence that the disulfide linkage in proteins should not be a primary target for mercury poisoning, since sulfur-bound mercury, CH_3HgSR, lacks the electrophilic properties to effect sulfur-bound bond cleavage. It was also established that these highly covalent mercurials will readily extrude sulfur from mercaptide containing metallo-enzymes.

Despite a wealth of information about the symptomatology of heavy metal poisoning, very little has been known concerning the mechanism of action of these metals at the cellular, membrane, and molecular level. Some of the heavy metals have potent effects on the nervous system. Research is being carried out to elucidate the mechanism of action of neurotoxic heavy metals on nerve and muscle cell membranes using electrophysiological techniques. Methylmercury has been chosen as the first toxicant to be studied because of its highly potent neurotoxic action and its serious environmental concern. A unique and extremely useful internal perfusion-voltage clamp technique for neuroblastoma cells has been developed and methylmercury has been found to have potent blocking action on membrane ionic conductance mechanisms. Studies of the details of this mechanism are continuing.

A study has been completed indicating that blood lead levels and air lead concentrations tend to corellate in human populations. In a remote area of the Himalayan Mountains, air lead content was negligible and the low blood lead levels were one log lower in this population than in Western populations. Therefore, the concept of "normal" blood lead levels generally accepted for Western populations is erroneous.

Standard treatments of lead intoxication by EDTA has also come under question. It has been suggested that a rapid redistribution of lead from bone to soft tissue occurs prior to lead elimination, subsequent to EDTA treatment. In some children with blood lead levels between 40 to 60 mg/dl, a 20-30% elevation of the blood lead occurs within 30 minutes after EDTA administration. The elevation in blood lead appears to be primarily an elevation in plasma lead concentration. Therefore, the redistribution of the lead into the plasma compartment following the administration of EDTA occurs more rapidly than the rate of renal elimination

of the EDTA-lead complex. Thus, more severe cases might better be treated by following up EDTA treatments with combination treatment of EDTA and BAL. Studies are continuing on these phenomena.

Lead

An assessment of the potential hazards to man from the inhalation of trace metals at concentrations likely to occur in the environment is being made. Using the rat lung as model, rats were exposed to aerosols of $NiCl_2$, $CdCl_2$ or $CoCl_2$, and sacrificed at measured intervals. Cobalt clearance from the IVPL closely followed a diffusionally controlled process. Nickel clearance was dependent upon the initial concentration of $NiCl_2$ instilled. Cadmium clearance was like Ni, being dependent upon the initial concentration of $CdCl_2$ instilled. In all cases, the clearance followed first order kinetics. The IVPL can be used to estimate the initial rates of removal of these metals and to test for alternative molecular mechanisms of removal, which are not possible for the in vivo technique. Data from the two techniques will be combined to develop a mathematical model of clearance of these heavy metals from the rat lung. The data presented represents the first information on the rates of removal of heavy metal ions at concentrations comparable to those encountered by man in his environment. The rates measured in rodent lungs are all first order and diffusionally controlled. Thus, it is highly unlikely that there will be major differences between the rates measured in rodents and those obtained in man. Because of the greater size of man to the rodent, there are of course differences in scale or absolute rates. The observation that heavy metals interact with the xenobiotic metabolism of the lung to alter both the chemical composition and rate of metabolism of a known carcinogen, benzo(a)pyrene, is highly important. That the heavy metals act directly upon the cytochrome P_{450} system is shown by studies with the IVPL measuring the O-demethylase activity.

Saturnine gout is one of the symptoms of lead poisoning and involves the metabolism of purine. A study is underway to assess the role of Pb^{2+} and other heavy metal pollutants on purine metabolism in order to discover the etiology of the gout. β aminoisobutyric acid (βAIB) is a catabolic product of nueleic acid degradation which was found to be increased from nine to twelve-fold in the urine of marmosets exposed to lead acetate in drinking water. (βAIB) is a catabolite of thymine found almost exclusively in DNA, although small amounts are found in transfer RNA. A urine sample that was obtained from a lead-poisoned human subject also had elevated (βAIB). Since there was no previously reported connection between destruction of DNA and lead poisoning, the marmoset model will be used to follow this interesting finding.

Scores on the McCarthy Scales of Children's Abilities, school reading tests, teacher ratings and several exploratory measures were obtained for urban black school age children first studied five years previously. These were related, for 63 children, to preschool blood lead, school age blood lead and FEP levels; and, for 34 children, to dentine lead. Most outcome variables were not significantly related to the lead variables. Preliminary analyses indicated that several of the McCarthy scales, including the critical General Cognitive Index and Verbal Scales, and the reading test were significantly impaired in higher lead level groupings. However, incorporating a brief measure of parent I.Q. into the analyses decreased variance associated with lead and led to a strong suspicion of the remaining significant results. Few investigators reporting positive effects have considered parent intelligence, known to be a major determinant of developmental

status. For this and other admittedly difficult methodological reasons, conclusions from prior studies are questioned.

A decrease in red cell P5N (CMPase) was found in children as a zero threshold manifestation of low level lead and may represent the earliest metabolic aberration of nucleotide metabolism. Chronic lead exposure, with a decrease in enzyme activity of the reticulocyte, is responsible for the accumulation of pyrimidine nucleotides in the mature red cell. Just as the zinc erythrocyte protoporphyrin (ZEP) reflects chronic lead exposure of the reticulocytes with defective heme synthesis in the antecedant three months, in a like manner, the appearance of cytidine and uridine phosphates reflects defective nucleotide metabolism probably combined with altered RNA synthesis in the developing reticulocyte. This will be the first cross age epidemiologic study of blood lead and ZEP with P5N and red cell nucleotide profile. Refinement of the methodology for both the enzyme and nucleotide assay makes it possible to offer another clinical index of lead exposure that appears more stable than blood lead since it reflects the sum total of exposure during red cell maturation.

The effects of perinatal lead exposure on brain growth, anatomy and physiological function is being determined. It was found that sequential transplantations of cell body and target regions of the CNS to the anterior chamber of the eye creates a functional, yet isolated, neuronal pathway which can be utilized to study the development of neuronal connections. The effects of adenosine and various derivatives were examined in the in vitro hippocampal slice preparation from rat, as well as the interaction of adenosine with various adrenergic agents in the hippocampal slice. The data suggest endogenously released adenosine is a potent modulator of hippocampal excitatory transmission. In contrast to biochemical studies, however, there is little electrophysiological evidence for the interaction of exogenously applied adenosine with alpha or beta adrenergic agonists. The effect of perfused norepinephrine on evoked potentials in the CAI of the in vitro rat hippocampus was determined and the results suggest that NE may interact with α-adrenergic receptors to decrease pyramidal cell excitability, and with β-adrenergic receptors to increase pyramidal cell excitability; the β effect may involve cAMP. A model for selective studies of regional and temporal effects of chronic lead exposure on brain development, based on intraocular brain tissue grafting, demonstrates the applicability of the grafting technique for studies of chronic low level lead intoxication. The method has revealed highly significant effects of lead on growth of certain selected brain areas.

In studies related to the neurotoxicity of lead during the embryonic stage, exposure of rats via the dam has produced subtle effects on neural development. Exposure via this model provides continuous, low-level exposure to lead (the probable exposure conditions of the pediatric population at risk) and demonstrated that this method of exposing the neonates to lead has physiological and neurochemical effects on the rat dam. Electrophysiological studies have shown that amygdaloid kindling was not substantially altered by neonatal lead exposure. The data, plus the results of MES and EST testing indicate that lead exposure selectively effects some seizure mechanisms and models, but not others. Neuropharmacological manipulations of awake rats bearing chronically implanted electrodes have increased our understanding of neurotransmitter systems in the hippocampus. Lead exposure has been reported to alter neurotransmitter systems. Neuropharmacological manipulations of electrode implanted lead-exposed and control rats may reveal lead-induced changes in neurotransmitter functions within the hippocampus.

A series of heavy metals have been tested for their teratogenic effects. Results are as follows: (1) The teratogenic effect of indium on the developing limb buds can be potentiated by the simultaneous exposure to a brief (45 minute) exposure of the mother to 39°C. These results indicate that maternal hyperthermia may be an important factor in the potentiation of certain subthreshold teratogenic environmental stimuli, (2) Cardiac malformations have been induced by copper in hampsters, (3) The sensitivity of the developing CNS to heavy metals is being tested. Thus far, no consistent lesion attributable to the heavy metals cadmium, lead, or arsenic have been determined by examining fetuses of exposed mothers just before birth, and (4) Similarly, reproductive performance in female hampsters who had been exposed to toxic levels of lead produce no significant levels of abnormal offspring.

The nature of proteins forming intracellular complexes with potentially toxic metals, e.g., lead, mercury, and cadmium, the functional changes in the cell containing intranuclear inclusions, and the effects of chelating agents in tissue culture to remove the metal are under investigation. Attempts are being made to determine if the proteins which bind intracellularly with the metals are synthesized specifically due to metal exposure. The studies involve both tissue culture experiments and whole animal experiments.

Selenium

For many years, the ability of selenium-sensitive plants to synthesize selenocysteine remained in question. The solution to this problem involved stabilizing selenocysteine before isolation. Experiments were carried out with Vigna radiata (mung bean), a selenium-sensitive plant, grown in the presence of selenate. Instability of selenocysteine was avoided by treatment of protein extracts with iodoacetic acid, prior to hydrolysis, in order to form the stable carboxymethyl derivative. The results have established that selenocysteine is present in the polypeptides of a selenium-sensitive plant. Excessive selenium, therefore, exerts its effect through replacement of cysteine by selenocysteine, altering normal enzyme activity.

Speciation of selenium-containing biomolecules is being followed in two algae species, Dunaliella primolecta and Chlorella sp., grown in a selenium-containing growth medium. Proteins, cellulose lipids, amino acids and carbohydrates were analyzed for Se by atomic absorption spectroscopy. The objective of the study is to determine the rate of incorporation of selenium into algal cells, as well as the rate of efflux from the cells, and relate the findings to the role of selenium in the food chain.

The assimiliation of selenium by selenium-sensitive, selenium-tolerant, and selenium-requiring organisms to elucidate the unique events involved in selenium utilization is being studied. It is hoped that a better understanding of the toxic role of this element as it effects both animal and human nutrition will be obtained. It has been determined that selenocysteine is formed from selenium in selenium-sensitive plants. This compound was isolated from plant polypeptides and therefore it has been concluded that replacement of cysteine by selenocysteine alters normal enzyme activity causing this sensitivity. In addition, a unique transport process for selenite as the initial step as an assimilatory pathway selected for this anion has been determined in microorganisms and the process is independent of the transport process for sulfate and selenate.

The role of heme degradation in the development of jaundice, enzyme characterization of the heme degradation pathway, and the effect of metal ions on biliverdin reductase activity are being studied. Heme oxidation is catalyzed by the microsomal enzyme heme oxygenase. The product of this reaction is biliverdin. In mammalian systems, this compound is reduced to the bile pigment bilirubin with biliverdin reductase catalyzing the reaction. It has been determined that heme oxygenase is a distinct microsomal enzyme system and that a variety of divalent elements as well as heavy metal ions regulate the synthesis and degradation of cellular heme and hemoproteins. Biliverdin reductase has been purified to homogeneity in a stable form. Activity studies suggests that the inhibition of biliverdin reductase by mercury was the result of interaction with essential sulfhydril groups of the enzyme and that the reversal of mercury mediated inhibition of the reductase by selenium in vivo was not the result of a direct interaction between the enzyme and selenium. In the rat model, a novel and apparently harmless procedure to prevent increased bilirubin formation in the development of jaundice has been found. It was discovered that zinc protoporphyrin can act as a selective inhibitor of heme oxygenase activity. The decrease in heme oxygenase by zinc protoporphyrin was not accompanied by alterations in any other measured cellular parameter.

Cadmium

Studies on the toxicology of cadmium have shown that hepatic metallothionein increased after treatment of rats with sodium iodoacetate due to an increase in synthesis of metallothionein. Rats exposed to twelve different metals by intraperitoneal injection of maximum tolerable doses indicated that zinc was most selective in producing an increase in metallothionein, whereas of the various other parameters measured, cadmium exerted the broadest effect. In rats, age distribution differences were shown in various tissues after administration of cadmium, with higher concentrations seen in the lung, bone, brain and blood of newborn when compared with young adults.

In humans exposed to cadmium, studies on metallothionein and urinary cadmium ratios suggest that such information may provide information of immediate diagnostic interest based upon an apparent relatively normal metallothionein/cadmium ratio over the range 0-40 years, with widely variant ratios indicating unusual conditions such as excess of free metal, presence of significant amounts of other metallothionein-inducing metals and unusual physiological functions.

Studies on cadmim toxicity at the molecular level have demonstrated that the H^+ gradient generated by substrate oxidation is labelized by interaction of the mitochondria with Cd^{2+} or PhAsO. Reversal of these effects by BAL, but not by monothiols is a finding that is consistent with the involvement of dithiols in the stable maintenance of a H^+ gradient across the mitochondrial membranes.

Radioimmunossay (RIA) is being used to elucidate metal interactions and various questions concerning certain metal binding proteins such as metallothioneine. Despite the fact that the existence of the cadmium metallothioneine complex has been known for many years, the precise role of the complex in metal toxicity is not yet well defined. It is not even known whether the binding action of cadmium is beneficial or harmful. RIA techniques are being used to quantify the variables involved and thus to develop new theories concerning this total process.

Exposure of kidney cells in culture to cadmium indicate a direct protective role

of the intracellular metallothioneine. In addition, it has been found that BAL or BAL-DTPA in combination may be an effective way of chelating cadmium and removing it from the body without affecting the kidney (the critical organ in cadmium toxicity).

Nickel

An investigation of the carcinogenic and mutagenic effects of metals and their compounds on in vitro systems has shown the following: 1) Pretreatment of Syrian hamster embryo cells with benzopyrene, an inducer of aryl hydrocarbon hydroxylase, potentiates the morphological transformation of Syrian hamster embryo cells induced by Ni_3S_2. The incidence of Ni_3S_2 transformation in cultures pretreated with benzopyrene was in some instances 10 fold greater than those transformations caused by similar exposure to either Ni_3S_2 or benzopyrene alone. 2) Ni_3S_2 treatment of Chinese Hamster Ovary cells caused the appearance of 2-3 6-thioguanine and 8-azoguanine resistant colonies (per plate, 7 X 10^6 cells plated) while untreated Chinese Hamster Ovary cells averaged 0.2 resistant colonies per plate for a similar number of cells at risk. Therefore, Ni_3S_2 displays very weak or no mutagenic activity in the mutagenesis system tested, and 3) carcinogenic activity of particulate metal compounds such as Ni_3S_2 is proportional to their cellular uptake. Cells actively phagocytized particulate Ni_3S_2 but did not take up amorphous NiS particles to a significant degree. The latter observation may help understand why specific metal compounds are carcinogenic.

Erythropoietin activity was determined in renal homogenates from rats receiving intrarenal injections of α Ni_3S_2 into the right kidney. Erythropoietin production was stimulated in the injected kidney without influencing similar activity in the contralateral non-injected kidney. Using sixteen different nickel compounds, the results indicated a correlation between the efficacy for induction of erythrocytosis and the carcinogenic activity of the compounds. Pathological examination of tissues from rats injected with α Ni_3S_2 showed pronounced erythroid hyperplasia in bone marrow, spleen and liver. The injected kidneys revealed a remarkable degree of hyperplasia of glomerular mesangial cells, a lesion not previously noted in kidneys of α Ni_3S_2-treated rats.

BIOMECHANISMS

The metabolism, disposition, and interaction of waterborne pollutants in fish is being studied to determine the persistence and bioaccumulation of several classes of chemicals, the rate at which these compounds are metabolized, and to gain a better understanding of the role of metabolism and the persistence in bioaccumulation of these hazardous materials in the food chain. The extent to which some of the chlorinated hydrocarbons induce xenobiotic metabolizing systems in fish, the basic mechanisms which underly various species sensitivities to the toxic effects of chemicals in the water supply is also being determined.

Studies devoted to understanding the interaction of various forms of liver microsomal cytochrome P-450 with the enzyme NADPH-P-450 reductase and epoxide hydrolase are under way. The substrate Warfarin is being used as a probe of the ability of different forms of P-450 to interact with the reductase because of the regional and stereo selectivity of the hydroxylations carried out by individual forms of P-450. Competition experiments indicate that different forms of P-450 interact preferentially with a limited amount of NADPH-P-450 reductase. These results strongly suggest that metabolism in vivo is controlled by the affinities

of the individual enzymes as well as their total concentrations. The preferential interaction of different P-450s with epoxide hydrolase has also been investigated in reconstituted enzyme systems using spectral techniques, kinetic determinations of transient times, and analysis of the metabolites of dichlorinated biphenyls. The different affinities of these enzymes for different compounds is being determined.

Glutathione regulation and its relationship to chemical toxicity is being investigated by studying (1) the protective role of glutathione during chemical intoxication, (2) how glutathione biosynthesis and glutathione content are regulated in various cell types during chemical intoxication, (3) the role of the cystathionine pathway in the biosynthesis of cysteine and glutathione in various cell types, and (4) the interorgan relationship for glutathione conjugate metabolism. The significance of these studies includes a better understanding of the important differences in mammalian organ cells in regard to the utilization of the cystathionine pathway for the biosynthesis of cysteine and glutathione. Parenchymal liver cells may be quite unique in having a very high capacity for cysteine biosynthesis via this pathway. The liver also appears to be a major producer for the maintenance of plasma glutathione levels. In turn, extracellular degradation of glutathione by the kidney allows the liver to be a major contributor to maintenance of plasma levels of cysteine and cystine. An extracellular thiol redox balance is implied in these interorgan relationships.

Studies are underway to determine the mechanism of alcohol potentiation of carbon tetrachloride hepatotoxicity, the metabolism of carbon tetrachloride, the role of phosgene in carbon tetrachloride-induced hepatotoxicity and the role of lipid peroxidation in carbon tetrachloride-induced hepatotoxicity. Ethanol potentiation of carbon tetrachloride toxicity occurs secondary to an ethanol-induced fast. In contrast, methanol, 2-propanol and tert-butanol potentiate carbon tetrachloride toxicity at doses that cause no loss in body weight. Investigating the mechanism of carbon tetrachloride reduction to chloroform, it was found that a one, rather than two electron reduction was most important. 2-propanol treatment was found to increase the metabolism of carbon tetrachloride to phosgene and carbon dioxide, the hydrolysis product of phosgene. Studies indicate a role for phosgene in carbon tetrachloride hepatotoxicity since 2-propanol treatment increases both the metabolism of carbon tetrachloride to phosgene and the hepatotoxicity of carbon tetrachloride.

A wide variety of xenobiotics are known to be hydrolyzed by mammals, however little is known about the substrate specificities of the enzymes involved. For this reason it is not possible to state which substrates compete for the same enzymes. Studies to elucidate the role of hydrolysis in toxicity have shown that a food additive, butylated hydroxyanisole (BHA), when administered in the diet of mice, boosts the whole body malathion carboxylesterase titer approximately 4-fold. Most of the boost could be accounted for by hepatomegaly, but some increase in specific enzyme activity in the microsomes was also observed. Assay for meperidine carboxylesterases by the same method showed that this enzyme does not occur in rat serum and, in rodent liver displayed a relatively low specific activity. Dietary BHA boosts the titer of meperidine carboxylesterases in the liver, primarily due to enlargement of that organ.

Delayed neurotoxicity induced by organophosphorus esters using the cat model has shown that administration of a single dermal dose of tri-o-cresyl phosphate (TOCP) ranging between 250 and 1,500 mg/kg caused delayed neurotoxicity. Severity

depended upon the dose and was characterized by ataxia progressing to paresis in animals given high doses. 100 mg/kg was not neurotoxic. Electrical manifestations of acute lower motor neurodysfunction were seen in 56% of the dosed cats as abnormal spontaneous activity consisting of positive waves, fibrillations and fasciculations. No significant changes were observed in neuromuscular junction transmission or in sciatic motor studies. The electrical abnormalities observed suggest a primarily motor axonopathy as the peripheral lesion in TOCP neurotoxicity.

Studies are underway to provide information which will be useful in predicting conditions in which non-target vertebrates may be unusually susceptible or resistant to the toxic action of pesticides and other chemicals as a result of exposure to pesticides. Upon repeated exposure to certain organophosphorus acetylcholinesterase (AchE) inhibitors, rats develop tolerance apparently through alterations of cholinergic receptor sites in the brain. Additional studies have shown that the development of tolerance to chronic organophosphorus treatment is, at least partly, mediated by a decrease of the muscarinic cholinergic receptors. Related studies showed that piperonyl butoxide (PB) non-competitively inhibited oxidative desulfuration which results in formation of the toxic oxygen analogues of the insecticides and that dibutylphthalate hydrolase is inhibited in vivo by otherwise non-toxic doses of at least certain OP compounds.

In order to study the effects of chronic sublethal cholinesterase (ChE) inhibition on the nervous system, rats were injected daily with either saline or paraoxon for up to 60 days. Plasma ChE activity was inhibited consistantly throughout the injection schedule by 15% and 30% following the administration of .05 and 0.1 mg/kg paraoxon respectively. In contrast, red blood cell enzyme activity exhibited a progressive inhibition with 20% and 40% inhibition measured after one injection of paraoxon and 65% and 70% inhibition following 60 days of injection. AChE activity was determined in EP and non-EP regions of muscle, whereupon histological examination of muscle specimens indicated a slow, but progressive development of skeletal muscle fiber necrosis paralleling the changes in EP enzyme activity.

A dynamic headspace sampling (DHS) technique was developed for trapping and concentrating volatile substrates and metabolites for subsequent analysis by gas chromatography and mass spectrometry. Using the DMS system, determination was made for the first time of the enzymatic consumption of volatile di- and trichloroethanes by oxygenated microsomal systems. For the four substrates investigated; 1,1 dichloroethane; 1,2 dichloroethane; 1,1,1-1,1,2-trichloroethane, substrated consumption correlated with the degree of dechlorination and DNA binding measured in other laboratories using radio-labeled substrates. Using the DHS procedure which allows for qualitative and quantitative analysis of very small quantities of volatile metabolites produced under enzymatic conditions, no volatile hydrophobic metabolites of 1,1,2-trichloroelthane were produced in microsomes under oxidative contitions.

Hydroxychlorodiphenyl ethers (HO-Cl_x-DPEs), predioxins and isopredioxins are the major contaminants of technical pentachlorophenol (PCP). The effect of HO-Cl_x-DPEs and PCP on erythrocyte membrane ATPase was shown to be inhibition of the Na^+, K^+Mg^{2+}-ATPase and stimulate the Mg^{2+}-ATPase, although differences in activity between the compounds were shown. Preliminary experiments employing spin-labels show that 2-HO-Cl_3-DPE has a marked ordering effect on sonicated vesicles from dipalmitoyl phosphatidylcholine below the phase transition temperature. Above

the phase transition temperature the effect is not as apparent. Studies on the effect of HO-Cl_x-DPEs on mitochondrial swelling demonstrated that non-energized high amplitude swelling is dose-dependent.

Studies to explain the mechanism of benzene toxicity have shown a difference in the susceptability of C57/B6 and DBA/2 mice to benzene. The difference is manifest as a greater reduction in red cell production as measured by reduced uptake of Fe^{59} into red cells following benzene treatment. Differences in the two strains included the urinary metabolites of benzene, the accumulation of water soluble metabolites of benzene in the various organs and the covalent binding of benzene metabolites in these organs. Hydrolysis of the conjugated metabolites of benzene in the urine followed by chromatography of the phenolic metabolites on HPLO showed that of the three major metabolites; phenol, hydroquinone and catechol; the catechol levels were equal in the two strains, but the C57/B6 excreted more hydroquinone, while the DBA/2 excreted more phenol.

Using mice whose hematopoietic reserve was eliminated by the combination of splenectomy and low dose Sr^{89} irradiation, four potential toxins were evaluated for their effects on the hemotopoietic system. The toxins used; busulfan, carbon tetrachloride, benzene and adriamycin, together with previously employed agents including lead acetate, polychlorinated biphenyl and benzo(a)pyrene, indicate that hematopoietic depression may be selective or preferential for one or more of the 3 formed elements studied. Resultant depression of peripheral counts may indicate pleuropotent stem cell damage and/or selective damage to the committed stem cells of one or more lineages.

Data obtained in the study of peripheral nerves of cats with ventral rhizotomies indicate that chronic endoneurial edema, increased endoneurial pressure and a chronically altered blood-nerve barrier are associated with a delayed paranodal demyelination. These findings are significant because few researchers have addressed the role of chronic endoneurial edema in the pathogenesis of nerve fiber damage. Present data support the contention that pathogenesis of numerous neuropathies may be attributed to breakdown of the blood-nerve barrier and chronic endoneurial edema. Studies in lead-intoxicated rats showed that lead can cause demyelination in the absence of an abnormally permeable blood-nerve barrier. Such findings do not support the hypothesis that endoneurial edema plays a major role in the pathogenesis of nerve fiber damage in lead neuropathy.

The atherogenic response of cockerels exposed to polycyclic aromatic hydrocarbon (PAH) carcinogens and other environmental pollutants has shown that 7,12 dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene, another PAH carcinogen and a prominent component of cigarette smoke, are all atherogenic in the test system employed. DMBA was shown to accelerate growth of pre-existing lesions rather than to induce formation of new lesions. Moreover, animals receiving DMBA from 20-36 weeks of age displayed lesion areas twice as large as those seen after any other exposure period. A study to determine the effect of chronic exposure to cigarette smoke on arterial lesion development showed that there was a marked trend toward an increase in lesion size in the smoking group compared to controls.

MUTAGENESIS-CARCINOGENESIS

Mice which are homozygous for the recessive gene dwarf (dw), in addition to impaired growth, show hypothyroidism. Hypothyroidism and diabetes have been associated with meiotic non-disjunction. Both hypothyroidism and diabetes are

believed to act via affecting the energy metabolism of the granulosa cells in the female, and the Sertoli cells in the male. Glycolysis and thus energy demand, is dependent upon these cells during gametogenesis in the male and including at least metaphase I of meiosis in the female. A comparison is being made of the magnitude of the non-disjunction response obtained with chemical mutagens with the one evoked by the health conditions mentioned above. Another point of practical significance will be the interaction between the two, that is, do the diabetic and/or hypothyroid mice react stronger after the administration of nondisjunction inducing chemicals?

The Ames Salmonella test is greatly enhanced in sensitivity by incorporating plasmid pKM101. Although this test system has a high predictive value for carcinogenicity in man, it does not detect all types and classes of mutagens. A total of 28 plasmids were screened for use in the Ames test. Other characterization of plasmids effects in Salmonella tester strains TA1535 and TA1537 include spontaneous mutation, UV survival, UV mutagenesis and chemical mutagenesis. Although none of the plasmids tested were proven to be superior to pKM101, different plasmids showed differences in spontaneous and UV-induced mutation frequency and on UV survival. Some plasmids are superior to pKM101 in enhancing UV mutagenesis but are less effective in enhancing UV survival, or vice-versa.

Studies are being carried out to determine whether different types of polycyclic aromatic hydrocarbons exert their mutagenic and carcinogenic effects by different mechanisms and specifically whether the mechanism involves activity corresponding to tumor promotion. Results to date show differences in the behavior of different polycyclic aromatic hydrocarbons toward V79 cell survival and mutagenesis, and suggests that the mechanisms of mutagenic and carcinogenic action of different agents of this type may be markedly dissimilar. The failure of 3-methylcholanthrene to affect chemically-induced mutation frequencies leads to the hypothesis that it has no intrinsic promoting activity *in vivo*.

Highly synchronized Chinese hamster cells *in vitro* are being used to study the basic mechanisms which lead to inactivation or mutagenesis of mammalian cells by alkylating carcinogens-mutagens. Using ethylnitrosourea (ENU) three types of genetic changes were shown to occur equally at any time in the cell cycle. This suggests that the different cellular biochemical processes occurring in the cycle do not influence mutagenesis by the agent ENU. Since the biochemistry of the cell cycle is not a deciding factor, the *in vitro* model for mutagenesis by ENU may be comparable to the *in vivo* response.

The strong correlation which exists between mutagenicity and carcinogenicity encourages development of mutagen-sensitive assays. The development of mutagen-sensitive Drosophila strains defective in precisely defined biochemical steps in DNA-repair processes is expected to be able to provide discriminating tester strains for determining the mutagenic capability and mechanism of action of environmental agents. Autoradiographic analysis of unscheduled DNA synthesis (UDS) and velocity sedimentation analysis of the molecular weight changes in treated vs. control DNA are being used to assess the cellular response of various *mus* mutants to DNA damage by UV or alkylating agents. UDS to UV and alkylation damage in $mus201^{D1}$ and $mei9^{A11}$ stocks which are excision deficient for UV damage confirmed a total lack of UDS capability for $mus201^{D1}$ with UV, and preliminary evidence suggests that a similar defect exists for $mus201^{D1}$ with MMS damage and $mei9^{A11}$ with UV damage.

An attempt is being made to develop a new short term assay in a mammalian cell

culture system utilizing a mammalian virus-cell system in which the replication and repair of carcinogen-treated SV40 viral DNA can be studied. Results indicate that neither spontaneous loss of adducted bases nor subsequent spontaneous DNA chain breakage is likely to be a decisive event in carcinogenesis. The same is true of small (ethyl) adducts at non-hydrogen-bonding ring nitrogens at DNA bases. Small adducts on hydrogen bonding groups cause misincorporation, which can result in point mutation, but this also is probably not a determining factor in carcinogenesis in every case. In addition it was found that large adducts on hydrogen-bonding groups do not cause misincorporation. Moreover there is evidence in the literature that they cause the termination of replication, in vitro.

The carcinogenic action of formaldehyde (HCHO) and hydrogen chloride (HCl) including the possible role of bis (chloromethyl) ether (BCME) formed from the interaction of HCHO and HCl are being studied. Rats exposed to lifetime regimes of single and combined chemicals are under observation. The weight gain in the exposed groups is substantially lower than that of the control groups. The highest weight loss was noticed in groups receiving the combined exposures, followed by the group receiving HCl alone. The rate of mortality was not significantly different among groups up to the thirty second week of exposure. However, at the end of sixty four weeks, the highest mortality was found in groups receiving the combined exposures (25%) and the least mortality was found in HCHO treated group (9%). The early deaths are mostly attributed to bronchiectasis and pneumonic lesions in the lungs. Fifty percent of the animals dying to date have been examined histopathologically. Striking damage was seen in the nasal cavity of the exposed rats. One animal in each group receiving the combined exposures and the formaldehyde alone developed a nasal swelling. On cross examination, these tumors appeared as opaque grey white masses which actually filled the nasal cavity and perforated the nasal bones. The swelling was first noticed at 403 days from an animal receiving the combined exposures (premixed), at 443 days from an animal receiving the combined exposures (not premixed) and at 421 days from an animal receiving formaldehyde alone. Histologically, all these tumors are classified as squamous cell carcinomas.

Acrylonitrile (VCN) and Potassium Cyanide (KCN) act by binding covalently to protein and through cyanide binding to heme respectively. Studies to date indicate that in contrast to KCN which mainly interacts through cyanide ion, VCN in addition to limited liberation of CN^-, may act by interfering with erythrocyte metabolism and energy production. This alteration may result in decreased supply of oxygen to different tissues, particularly those which are very sensitive to oxygen threshold such as brain. This finding might imply a role in VCN neurotoxicity. Moreover, data indicate that there is species difference in VCN toxicity and metabolism and suggest that VCN is metabolized to cyanide via a mixed function oxidase enzyme system (MFO). A study to determine whether brain tissue is capable of metabolizing VCN to Cyanide indicated that acrylonitrile (VCN) is metabolized to CN^- by brain tissue. The apparent greater affinity of VCN for cytochrome P-450 of brain tissue compared to liver tissue may be an important factor in the central nervous system toxicity of VCN.

The environmental health aspects of the reactions of ozone with organic compounds are being studied. The oxidant carbonyl oxide was shown to be capable of converting phenylalanine to tyrosine and phenanthrene converted to 9,10-oxide. The results of these studies provide growing support for the proposal that carbonyl oxides are excellent models for the MOX enzymes which require flavin coenzyme. The observation of an intramolecular oxidation brings the study very close to the

physical aspect of the enzymatic oxidation, namely oxidation of a bound substrate. The observed linear free energy relationship establishes the electrophilic character of the oxidation in this model system and tends to confirm earlier suggestions regarding the electrophilicity of the enzymatic oxidation. The results to date provide further support for the earlier postulate (and fear) that partial or complete activation of polycyclic aromatic hydrocarbons (including precarcinogens) can occur without intervention of metabolic processes. Furthermore, the conditions for accomplishing this activation are likely to be present in polluted atmospheres.

To improve the prevention of human neoplastic disease it is vital to evaluate the mechanisms by which environmental carcinogens initiate the onset of human cancer. An understanding of the pathogenesis of human malignancy requires answers to current questions concerning the relationship between mutagenesis and carcinogenesis. By characterizing DNA repair enzymes in human liver, the question of how human cells control mutagenesis by the capacity to maintain the correct nucleotide bases in the human genome should be answered. Three isozymes of human liver AP endonuclease were completely resolved by column chromoatography. Each isozyme was then purified to apparent electrophoretic homogeneity as judged by denaturing and non-denaturing polyacrylamide gel electrophoresis. In order to minimize the possibility that the three isozymes arise from proteolysis of a single protein, all buffers contained 10^{-4} p-toleune sulfonyl chloride, a powerful inhibitor of proteolysis. Three isozymes of AP endonuclease were observed in the presence or absence of the proteolytic inhibitor. Hence, it is unlikely that proteolysis is responsible for the presence of three isozymes of AP endonuclease. Studies with the homogenous preparations of the three isozymes indicate they may be distinguished on the basis of molecular weight, thermal stability, metal ion cofactor kinetics, and substrate kinetics. AP endonuclease III was significantly more thermostable than AP endonuclease I or II. Studies with depurinated X174 RFI DNA indicated all three AP endonuclease isozymes incise depurinated DNA at all of the AP sites and at no other sites. DNA exonuclease activity was not detectable in AP endonuclease I, II or III.

Patients with the autosomal recessive disease ataxia telangiectasia are known to show predisposition to cancer. Their fibroblast cells in culture are more sensitive to gamma irradiation than normal. Cells from four different genetic backgrounds were used and examined for the activities of five classes of enzymes presumably involved in the repair pathway. These include (a) superoxide dismutase-catalase which scavenges the harmful molecular oxygen generated by ionizing radiation, (b) endonuclease specific for gamma radiation damaged sites in DNA, (c) N-glycosylase which catalyzes the removal of damaged or modified bases, (d) AP-endonuclease, an enzyme that hydrolyzes phosphodiesterase linkages adjacent to an apurinic or apyrimidinic site in the DNA, and (e) ligase, the essential component for ligation in both repair and replication synthesis. In addition, the ability of these cells to undergo unscheduled DNA (repair) synthesis was also examined along with normal cells. The results show that the defect in AT weakened the cellular capacity to remove bases and nucleotides damaged by ionizing radiation. In at least two cases, repair synthesis were also reduced. The cells ability to scavenge free radicals or to rejoin breaks in the sugar-phosphate backbone of DNA, however, was apparently not affected.

Two major categories of CHO mutants were used to understand mechanisms for UV resistance, thus repair pathways for UV damages. Three mutants in this category were studied in detail. Two of them, UV-1 and UV-2, have remained stable and

one has reverted to wild type UV sensitivity. These mutants differ in their relative sensitivity to the UV mimetic drug, mitomycin C and to the postreplicational repair inhibitor caffeine, after UV exposure. They also differ in the rate of unscheduled DNA synthesis and initiation of excision repair. Karyotypic analysis showed no obvious damage in the chromosome number. The mutants showed a prolonged time for DNA doubling but the cell doubling time was similar to the wild type. It is inferred from these observations that UV-1 acquired its resistance to UV through a more effective postreplication repair, while UV-2 survived through more efficient excision. Furthermore, the apparent independence of resistance to UV and mitomycin C suggests that various repair pathways are involved in CHO cells to remove their damages to DNA.

TEST DEVELOPMENT

The neurotoxicity of various organic intermediates on the central nervous system of primates is being studied. Acrylamide is the model compound being used to study morphological and electrophysiological techniques utilized to pinpoint the most vulnerable areas of the peripheral and central nervous system and to monitor the degree of recovery in the living animals. Baseline morphological data has been established as has sensitive noninvasive techniques for assessing the earliest onset of brain damage associated with neurotoxins.

An in vivo technique for evaluation of the somatic and mutagenic effects on human cells from exposure to pollutants is under development. Human and mouse bone marrow cells and peripheral blood cells are grown in fusion chambers, implanted in host animals, and are then exposed to pollutants via several routes. Cytogenic analysis for chromosome aberrations and sister chromatid exchange are being made on the cells cultured in the diffusion chambers and on the bone marrow cells of the host animal. The system is being validated by studying marrow from patients before and after chemotherapy, both tests being evaluated in a similar manner. Dominant lethal mutation analyses are also made on animals in selected treatment groups to compare the different mutation test systems.

Two assay systems are being explored to test the mutagenic or carcinogenic potential of compounds in the environment. The first system is a host-mediated assay by culturing mammalian calls in diffusion chambers (DC) and implanted into mice. The induction of point mutations, chromosome aberrations and sister chromatid exchanges (SCE) of the target cells in DC after the hosts are given the compounds under test are being used as indicators for mutagenicity or carcinogenicity. The second system is a liver homogenate mediated assay by incubating DC filled with the rat liver extract, cofactors and a compound under test with mammalian cells in vitro. The same indicators as described for the first system is being used. The two systems have been tested for screening mutagenic and/or carcinogenic potential of environmental compounds especially those that need to be metabolically activated. The systems are simple, sensitive and economical in time and cost. The results suggest that the cancer promoter-TPA and inhibitor-retinoids themselves had no direct effect on genetic materials but they may indirectly alter the effect of a mutagen or carcinogen. The ability of a total 17 organophosphorus pesticides (OPP) on the induction of SCE and cell cycle delay were studied. Based on the data as well as those reported in other systems, it would appear that the OPP which induce no SCE increase and no or slight cell cycle delay could be considered as good candidates to substitute the pesticides that have been found to be harmful to the environment.

The development of mutagen-sensitive Drosophila strains defective in precisely defined biochemical steps in DNA repair processes may provide discriminating tester strains for determining (1) the mutagenic capability of various environmental agents and (2) the mechanism of action of agents found to have significant mutagenic/carcinogenic activity. Employment of the Sex Linked Recessive Lethal (SLRL) test to monitor spontaneous mutagenesis in the excision-defective strains indicated that various mei-9 alleles in homozygous females enhanced the spontaneous mutation rate of X chromosomes derived from mature sperm whereas mus(2)201 females did not. For induced mutagenesis, mei-9 females show a substantial enhancement in the SLRL rate observed for mature sperm treated with EMS(2X), MMS(6X) and MNU(7.5X). Similarly, preliminary data for the F1 somatic mutagenesis experiment using the white eye color single gene system further support the observation of limited EMS hypermutability for the mei-9 locus. These data taken together support the notion that the mei-9 locus is active in repairing both spontaneous and alkylation-induced lesions occuring in male sperm. Similarly, for EMS, the excision-defective mus(2)201 locus appears to play a role in the repair of alkylation-induced premutagenic lesions. Preliminary data on the role of mei-9 repair activity during spermatogenesis and in the larval stages indicate the feasibility of study of such mutagenesis systems but no conclusive data are yet available.

Mutagenesis is being examined in germinal and somatic cells of the higher eukaryote and argiosperm Zea mays in order to develope a genetic assay for use as a monitor for mutagens in the environment. Experiments have shown that chronic exposure to low concentrations of mutagens is more efficient in the induction of point mutations in both germinal and somatic cells.

Studies are underway to develop a eukaryotic Ames type mutagen monitor at the waxy locus in Hordeum vulgare. Thirty mutants at the waxy locus have been induced by sodium azide and gamma rays. The mutants fall into nine allelic groups and have mapped to one locus controlling starch disposition. Spontaneous reversion rates for the different mutant alleles vary between 6×10^{-5} and 4×10^{-4}. Heteroallelic recombination frequencies among a dozen alleles ranged from 2×10^{-5} to 2×10^{-3}.

Colonies of Chinese hamster cells which are mosaic for glucose-6-phosphate dehydrogenase (G6PD) activity are being employed as a test for induction by mutagenic agents. The effect of replication state of the G6PD gene was investigated using synchronized cell populations. Cells exposed to either UV light or ethyl methane sulfonate at the G_1 cell cycle stage still produced mosaic colonies. This result indicates that mutation at one of two replicated G6PD genes is not the sole cause of the sectoring phenomenon. The possibility that mosaic colonies may be produced by mutation in one cell of a multicell aggregate was investigated by carefully breaking up the cell suspension using a 20-gauge syringe needle. No observable reduction in the frequency of mosaic colonies was observed after this procedure, indicating that aggregation is not a primary cause of mosaic colonies. The stability of $G6PD^-$ cells in the mosaic colonies has been examined by isolating cells from daughter colonies on nylon cloth replicas. To date, all mosaic colonies give rise to stable populations of pure mutant and non-mutant cells.

ORGANIC CHEMICALS

The biotransformation of aliphatic nitriles such as acrylonitrile is being studied to define the molecular events responsible for their toxicities. Specifically,

the mechanism of hematotoxicity of acrylonitrile, the compound's brain metabolism, and the role of biliary excretion and glutathione depletion is under investigation. It has been determined that, in contrast to potassium cyanide which mainly interacts through the cyanide ion, acrylonitrile, in addition to limited liberation of cyanide ion, may act by interfering with erythrocyte metabolism and energy production. This alteration may result in a decreased supply of oxygen to different tissues, particularly those which are very sensitive to oxygen threshold, such as the brain. Studies indicate that acrylonitrile is metabolized to cyanide ion by both liver and brain tissue. However, a greater affinity of acrylonitrile for brain cytochrome P-450 compared to liver tissue may be an important factor in the central nervous system toxicity of the compound.

The compound 2,4- dithiobiuret (DTB) is being used as a model for environmental agents which induce skeletal muscle weakness and a system for evaluating DTB toxicity is being tested on other compounds that produce neuropathologies in animals and man by yet unknown mechanisms. The ultimate goal is to develop antidotes for the various agents. Experimental conditions have been established for producing muscle weakness in a reproducible fashion. Daily DTB doses have been determined which will cause rotarod test insufficiencies in a reproducible manner.

The metabolism and activation of environmental polycyclic chemicals is being studied. The objective is to elucidate the proximate and ultimate carcinogens formed metabolically from the environmental polynuclear aromatic hydrocarbons. By examining a range of polycyclics, the theory of "Bay-Region" diol epoxides can be tested. Certain of these compounds have been found to indeed form diol epoxides. However, others such as 2- and 3-methylfluoranthene are weak carcinogens which do not have a "Bay-Region." Nevertheless, they have oxidizable regions and have been established as proximate mutagens. The mechanistic studies are therefore extremely important to establish a foundation for evaluating the influence of environmental modifiers on the metabolic activation or detoxification of these carcinogens.

The metabolism of di-, tri-, and tetrachlortethanes by rat liver microsomes is being studied to determine the mechanism of oxidative dehalogentation. These studies are complicated by the fact that the physical and chemical properties of the compounds and potential metabolites either caused them to be highly volatile, hydrophilic, or chemically reactive. For these reasons, specialized analytical techniques for use in sampling and analyses are being developed. The identification of the metabolic products will be very useful in the understanding of the toxicities and metabolisms of this important class of chemical compounds.

The relationship between the biotransformation of haloethylenes to reactive metabolic compounds and haloethylene induced acute liver injury using pretreatments which modulate both the metabolism and hepatotoxicity of these compounds is being studied. The types and rates of urinary excretion of soluble metabolites produced by animals with varying sensitivity to trichloroethylene were compared. These experiments indicated that the metabolic processes of the experimental animals were saturated by low levels of trichloroethylene. However, pre-treatment with phenobarbital or Aroclor led to differences in metabolic patterns such as alterations in the rate of excretion of free trichloroethanol and trichloracetic acid and in their ratios. Glucuronide conjugation was also inhibited, indicating the greater likelihood of cell injury due to covalent binding.

The mechanism underlying the atherogenic response from polycyclic aromatic

hydrocarbons (PAH) is being studied to determine if these compounds might be contributing to the high level of heart disease in the United States. PAHs are present in tobacco tar and smoke, smoked foods, coal and diesel exhaust, in addition to many other special environments such as charcoal broiling, etc. A known PAH, 7,12 dimethylbenz(a)anthracene(DMBA) has been used in an animal model system to begin to clarify the specific steps involved in the enhancement and acceleration of arterial lesions.

The role of the enzyme epoxide hydrolase in the metabolism of toxic haloalkenes and acrylonitrile is under investigation. It was determined that the suspected carcinogen, acrylonitrile, is converted to its oxide by cytochrome P-450. However, the epoxide was a poor substrate for epoxide hydrolase. Other epoxides of these compounds are being synthesized for similar testing.

Attempts are being made to determine whether the formation of muconaldehyde plays a role in benzene hematotoxicity. From its chemical structure, the compound appears to be a reasonable metabolite of benzene. Confirmation studies to determine this metabolic route are underway. In addition, various isomers of muconaldehyde have been synthesized and it has been found that the toxicities to human erythropoietic precursor cell cultures is very high. These studies have a direct bearing upon the unknown injury to bone marrow cells of the industrial important compound benzene.

Dichlorobenzidine (DCB) is widely used in the manufacture of azo dyes and is a curing agent in the production of polyurethane foam. It is known to be a potent carcinogen in animals and therefore assumed to be carcinogenic to man. The metabolic fate and the mechanism by which it produces its carcinogenic effect are under investigation. From radio-labeled studies it has been determined that DCB or its metabolites undergo enterohepatic circulation. Since hydrolysis of biliary conjugates of drugs is a prerequisite for intestinal absorption and enterohepatic circulation. The conjugated metabolites from the bile of DCB treated rats are most likely hydrolyzed in the gastrointestinal tract by bacterial action before reabsorption. Using animals with ligated bile ducts, none of the radio-labeled DCB was found in the feces indicating that no process other than biliary excretion resulted in the elimination of the material into the gastrointestinal tract. In addition, binding studies of DCB to polyribonucleotides has indicated that these aromatic amines show a marked preference for reaction with guanine in the experimental animals' genetic material.

The metabolism and toxicity of carbon tetrachloride and benzene, the role of sulfhydryl compounds in carbon tetrachloride toxicity and the mechanism of alcohol potentiation of carbon tetrachloride toxicity is being investigated. It has been demonstrated that the ethanol potentiation of carbon tetrachloride toxicity occurs secondary to an ethanol-induced fast. This finding was supported by the observation that fasting also potentiated carbon tetrachloride toxicity. In contrast, methanol, 2-propanol, and tert-butanol potentiate carbon tetrachloride toxicity at doses that cause no loss of body weight. No evidence suggested a role for lowered glutathione levels in 2-propanol potentiation of carbon tetrachloride toxicity was found. However, glutathione depletion does appear to be involved in the potentiation of carbon tetrachloride toxicity by ethanol or fasting. Phosgene has been identified as a metabolite of carbon tetrachloride, playing a role in the compound's hepatotoxicity. It has been found that 2-propanol treatments specifically increases the metabolism of carbon tetrachloride to phosgene and carbon dioxide. These studies will help elucidate the mechanism

by which chemicals produce liver damage which is a common toxic effect of halogenated solvents.

The effects of three different doses of PCBs fed to lactating rats during days 1-9 of lactation on reproductive ability, mating behavior and activity and learning of the female offspring was determined. The results indicate that (1) The percent weight gain in the litters was significantly less in the offspring exposed to the two higher doses of PCBs when compared to normal controls. By 75 days of age, no significant differences in body weight among the offspring of the treated and control groups were noted, (2) Vaginal opening was delayed in females exposed to higher doses of PCBs when compared to controls, and (3) It appeared that PCB-exposed females were more reluctant to mate than either normal controls or undernourished controls. Data from the pregnant females indicated that in the young females, exposure to the highest dose of PCB, had a marked effect on implantation but not on ovulation.

Structure-activity relationships and delayed toxic activity were determined for a series of O,O,S-trialkyl phosphorothioates, O,S-dialkyl alkylphosphonothioates and arylphosphonothioates. Signs of intoxication include weight loss, diarrhea, excessive urination and bleeding from nose and mouth. O,S-Diethyl methyl-, O,S-diethyl ethyl-, and O,S-dimethyl ethylphosphonothioate showed post 48 h toxicity. Results to date indicate a non-cholinergic mode of action for both trialkly phosphorothioate esters and dialkyl alkyl- or aryl-phosphonothioate esters. Metabolism and pharmacokinetic studies coupled with antagonism studies have provided basic information which may now be used to determine the site(s) of action in delayed toxicity.

The chemical events in the metabolism of toxic haloalkenes and acrylonitrile and understanding of the role of the enzyme epoxide hydrolase in the metabolism of these olefins and related compounds is being explored. Previous studies utilizing purified epoxide hydrolase and alcohol dehydrogenase as specific inhibitors of the binding of 2-haloethylene oxides and 2-haloacetaldehydes indicated that 2-chloracetaldehyde is the metabolite of vinyl chloride responsible for the binding of products to protein. Similar results were found in the case of vinyl bromide. With both vinyl bromide and vinyl chloride, the epoxides are the metabolites which appear chiefly responsible for DNA binding. Thus a difference exists in which metabolites are bound to different classes of macromolecules. Studies with isolated rat hepatocytes indicate that both 2-chloroethylene oxide and 2-chloroacetaldehyde can penetrate the cell membrane to alkylate extracellular DNA. These results have relevance to the observation that hemangiosarcoma is a tumor of the endothelial cells, which are largely incapable (in contrast to hepatocytes) of metabolizing vinyl halides. 1,1,2-Trichloroethylene oxide was synthesized and its decomposition ($t_{1/2} \leq 1$ min in aqueous systems) studied. The data suggest that cytochrome P-450 converts trichloroethylene to chloral without the intermediary of an epoxide. Other data indicate that the steady-state level of the epoxide reached in microsomes from tumor-susceptible hybrid mice is 4-5 times as high as in the case of rats, which are not susceptible.

3,3'-dichlorobenzidine (DCB) is widely used in the manufacture of azo dyes and as a curing agent in the production of polyurethane foam. DCB is known to be a potent carcinogen in animals and is currently regarded by the U.S. Occupational and Health Safety Administration as being carcinogenic to man. In spite of the potential carcinogenicity of DCB, there is extremely limited information available

with respect to its metabolic fate or the mechanism by which it produces its carcinogenic effect.

Disposition studies on DCB suggest that DCB or its metabolites undergo enterohepatic circulation. The ability of DCB or its metabolites to undergo enterohepatic circulation is of interest since it will increase their residence time in the body and potentiate carcinogenic action of the chemical. This phenomenon may explain a slow decline of radioactivity from plasma, liver, kidney, and lung. Studies on the interactions of DCB with polyribonucleotides have shown that DCB or its metabolites were most reactive towards poly G, suggesting that quanine is the main site for DCB binding in nucleic acids. These findings are consistent with previous studies demonstrating that aromatic amines show a marked preference for reaction with quanine. Moreover, pretreatment of rats with DCB caused 45% and 20% increases in hepatic microsomal P_{450} content. Other salient effects observed in hepatic microsomes from DCB pretreated rats indicate that DCB may be classified with the polycyclic hydrocarbons in terms of induction characteristics.

The significance of phosphorylation in organophosphate-mediated β-glucuronidase release from the rat liver was studied. It was found that the elevation of rat blood β-glucuronidase caused in vivo by O,O-dialkyl O-phenyl phosphates and phosphorothioates correlated well with the electron-withdrawing tendency (σ^-) of leaving group substituents indicating the importance of a phosphorylation mechanism in the enzyme release. Hydrophobic bonding of these compounds may facilitate the phosphorylation since hydrophobicity (π) of substituents also correlated with the enzyme release. SKF 525-A decreased the elevation of β-glucuronidase by parathion through the suppression of paraoxon production . Pretreatment of rats with phenobarbital or DDE resulted in lower and delayed enzyme release caused by parathion.

A pharmacokinetic model to predict the disposition of ^{14}C labeled 2,5-hexanedione (2,5-HD) was developed. The physiological pharmacokinetic model for the body disposition of 2,5-HD has been formulated and verified for its basic elements. The blood, plasma, liver, kidney, muscle, fat, lung, skin and especially sciatic nerve, (lumbar) spinal cord, and some brain tissue levels can now be predicted for ^{14}C radiolabeled 2,5-HD. The radiolabeled drug was measured by liquid scintillation techniques after either wet oxidation or combustion of the tissue samples. The tissue concentration showed the expected sharp rise and slower decline by metabolism and/or clearance of the drug. However, after 24 hours, the tissues, especially the nerve tissue and spinal cord, appeared to retain a significant level of radioactivity, which could indicate an incorporation of the drug and/or further metabolites. It was found from the $^{14}CO_2$ expired that about 90 (mol)% of the drug was apparently metabolized by 8-12 hours, with a corresponding molar amount of an unknown metabolite excreted in the urine (very little ^{14}C appeared in the separation of the metabolite(s), since the 2,5-HD is apparently not the final metabolite of, e.g., hexane.

The role of glutathione acting as a protective agent during chemical intoxication is under investigation. The significance of these studies includes a growing understanding of important differences in mammalian organ cells in regards to the utilization of the cystathionine pathway for the biosynthesis of cysteine and glutathione. Parenchymal liver cells may be quite unique in having a very high capacity for cysteine biosynthesis via the cystathionine pathway. The liver also appears to be a major exporter organ for maintenance of plasma glutathione levels. In turn extracellular degradation of glutathione by the kidney allows the liver

to be a major contributor to maintenance of plasma levels of cysteine and cystine. An extracellular thiol redox balance is implied in these interorgan glutathione cyst(e)ine relationships. Evidence is accumulating which indicates that the metabolism of glutathione conjugates is more complex than originally thought. The use of AT-125 as an inactivator of γ-glutamyl transpeptidase is a power tool to examine the location and relative roles of organ activity in degradation of these conjugates.

Both nitro and amino aromatics are recognized to be highly mutagenic and the known carcinogenicity of the amino aromatics is well known. Using a test system employing S. typhimurium with and without rat liver homogenate, studies were carried out on a series of nitrobiphenyls and nitronaphthalenes, their substituted derivatives and the corresponding amines. The findings show that Ortho-methyl substitution in nitrobiphenyls and nitronaphthalenes usually inhibits mutagenicity, while an enhanced effect was observed in the corresponding aminobiphenyls and aminonaphthalenes. This result indicates that the steric or electronic factors required for N-oxidation or nitro reduction are different for both classes of compounds. Both 4-amino and 4-nitrobiphenyl are potent mutagens while the 3- and 2-isomers in both cases were inactive. This result suggests a possible formation of a common active intermediate from both nitro- and aminobiphenyls.

Attention has been devoted to understanding the interaction of various forms of liver microsomal cytochrome P-450 (P-450) with the enzymes NADPH- P-450 reductase and epoxide hydrolase. The substrate warfarin has been used as a probe of the ability of different forms of P-450 to interact with the reductase because of the regio- and stereoselectivity of the hydroxylations carried out by individual forms of P-450. The results of P-450 competition experiments indicate that different forms of P-450 interact preferentially with a limited amount of NADPH-P-450 reductase. These results strongly suggest that metabolism in vivo situation is controlled by the affinities of the individual enzymes as well as their total concentrations. The preferential interaction of different P-450s with epoxide hydrolase was also investigated. The results indicate that, as in the case of NADPH-P-450 reductase, epoxide hydrolase has an inherently greater affinity for 5,6-naphthoflavone-induced P-450 than for phenobarbital-induced P-450. These findings may contribute to our understanding of the implications of enzyme interactions in the metabolism of potentially harmful xenobiotics.

A project to investigate the molecular mechanism of noncholinergic effects of organophosphorus insecticides on the liver is in progress. The rapid uptake of parathion by isolated hepatocytes and chromatographic elution of infused parathion from perfused liver reflect rapid and almost complete uptake of parathion by periportal hepatocytes from the infused solution. Autoradiography was employed to provide visual evidence for this uptake. Since parathion and paraoxon infused into the liver during perfusion are absorbed nearly exclusively by hepatocytes near afferent vessels, it should be possible to estimate the metabolic capability of those cells by analysis of metabolites produced before the chromatographic migration of the absorbed organophosphates. Such analyses are in progress.

The detoxification mechanisms utilized by insects are under investigation to better understand insecticide action and insect resistance to pesticides. Several studies have indicated that differences in detoxifying enzymes within the same species of insects can cause marked differences in sensitivity to various pesticides. This phenomenon may be of importance in insect control. For example, it was found that different enzymes are induced by different compounds in the

diets of insects and the knowledge of this phenomenon may be useful in the development of insect control strategies.

The effects of pesticides and other environmental chemicals on renal biochemistry and function during development and in mature animals is the objective of a current project. The polybrominated biphenyls (PBBs) have produced a variety of effects including alterations in the endocrine system and in the activity of microsomal mixed function oxidases. Because steroid hormones are substrates for MFOs, the effects of PBBs on the endocrine system may be a consequence of enhanced steroid hormone catobolism. It was determined that effects of PBBs on progesterone metabolism were dependent upon sex. In addition, Dinoseb, a derivative of dinitrophenol, has been shown to produce alterations in kidneys of mice and rats. The postnatal morphology and functional capacity of the kidney following prenatal treatment with Dinoseb in rats showed marked renal alterations. The kidneys had dilated tubules and excessive mesenchymal tissue when examined perinatally. Also the antioxidant food additive butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were evaluated for possible effects on renal function. It was demonstrated that these antioxidants have a specific depressive action on organic ion transport in the kidney.

The toxicokinetics of carbofuran poisoning is being measured in the adult female housefly. The biological effects of these concentrations are likewise under investigation and the mechanisms of these effects are being sought. Pyrethroids and its analogs seem to produce an increase in miniature postsynaptic potentials in muscles which correlates with toxicities. Aconitine is thought to increase the permeability of sodium in the resting nerve membrane. It also mimics the action of pyrethroids on motor nerve terminals. All these studies are ultimately directed toward understanding how the central nervous system responds to insecticide poisoning. Through this understanding the search for selective pesticides can be facilitated.

The biochemical events associated with the estrogenic actions manifested by certain chlorinated hydrocarbons are being characterized. It has been confirmed that several isomers of DDT are estrogenic. It has been found that chlorinated hydrocarbons inhibit the estradiol-mediated induction of uterine ornithine decarboxylase (ODC). The possibility that these compounds render estradiol inactive by increasing its rate of hydroxylation now appears untenable because there was no corollation between ability to inhibit induction of ODC and the ability to induce hyroxylation of estradiol.

The uptake, metabolism and excretion of Kepone in man and experimental animals is under investigation. A practical and reproducible assay for Kepone has been determined to aid in this study. Also, the role of various plasma lipoprotein fractions in mediating uptake of Kepone into the liver is being determined. Finally, an animal model (the gerbil) has been determined to be an inexpensive and practical model of human Kepone metabolism. These results relate to studies of Kepone toxicities specifically, and other chlorinated hydrocarbons in general.

Although large amounts of hexachlorobenzene (HCB) are used in agriculture and industry and the chemical tends to persist in the environment, there is little information on the effects of long term exposure to the chemical. Prominent in the toxicity of HCB and other chlorinated benzenes is the ability of these chemicals to cause porphyria in experimental animals and man. The mechanisms responsible for the development of porphyria in animals exposed to HCB and related compounds is not known. There is strong circumstantial evidence that HCB is

converted to highly reactive metabolites by mixed function oxidase systems in the liver and other tissues. Covalent binding of such HCB metabolites to protein and other cellular nucleophiles may play a central role in the development of porphyria. An investigation of HCB-induced porphyria is being made to understand its mechanism and possibly the way other chlorinated benzene type compounds cause porphyria.

It is being determined whether the acute necrotic muscle lesions occurring after exposure to acetylcholinesterase (AChE) inhibitors is a direct consequence of AChE inhibition and therefore produced by all anticholinesterases and insecticides of this type or if the effect is due to a direct toxic effect on the muscle. Experiments have indicated that AChE inhibitors, both carbamate or organophosphate classes, cause similar presynaptic as well as postsynaptic changes. If AChE activity is inhibited for 2 hours, there is no difference in the pre- and postsynaptic phenomena observed. It is now being determined whether or not the denervation observed in approximately 20% of the muscle fibers is the cause of temporary adaptation (loss of necrosis).

The outstanding teratogenic signs produced by organophosphate insecticides are skeletal malformations, such as micromelia, bent joint, abnormal beak, and short neck in developing chick embryos. Therefore, the effects of the agents on the development of cartilage, bones, and joints in relation to mechanism of their teratogenicity are being studied. It has been found that instead of development of ossified bones, cartilage disappeared in the middle of each long bone from day 9 of development. Other malformations were also observed. Malathion injected into 24 96-hour chick embryos caused abnormalities in the formation of the nervous system, somites, and major blood vessels, with the youngest injected embryo showing the most extensive and severe defects.

A practical model is being developed using the cat, to study the delayed neurotoxicity induced by organophosphorus esters. An integrated approach will correlate the clinical condition, neurological examination, electrophysiological changes, electromyographic measurements, histopathological alterations in the central and peripheral nerve tissues, biochemical parameters such as cholinesterases and acid phosphatases in the brain and plasma, and neurotoxic esterase in the brain of cats with model compounds. Single doses show a uniform dose response curve. Animals sensitive to delayed neurotoxicity induced by organophosphorus esters metabolize and eliminate these neurotoxic chemicals to a much lesser extent than those species which are not sensitive. Preliminary results indicate that the electromyographic tests may be used to study delayed neurotoxicity in humans exposed to these compounds.

EPIDEMIOLOGY

A major effort to determine whether the natural history of chronic obstructive respiratory disease (CORD) and functional respiratory impairment correlates with long-term exposure to photochemical oxidants by examination of residents in four demographically similar communities in Los Angeles County exposed to different types and levels of air pollutants is continuing. The results of analysis of data suggest that both current smokers and never smokers residing in the area exposed to high levels of photochemical oxidants have poorer lung function than residents exposed to low levels of pollutants or to high levels of SO_2, particulates and hydrocarbons. The primary site of impairment appears not to be the small airways as might be predicted from smoking studies.

A multidisciplinary investigation of the probability/statistical nature of environmental health problems has touched upon a number of statistically oriented approaches. In one such study fifteen patients with osteosarcoma were treated with transfer factor from household contacts. Of the fifteen, eight had evident lung metastases at the start of the treatment. Their survival was comparable to that of metastasis-free patients of historical series. The survival of the seven patients without obvious metastases was remarkably better than would be expected from historical records on some 2000 patients. Transfer factor apparently induces lymphocytic infiltrates in tumors.

By formulating epidemiological concepts of risk, survival, competing risk and underlying and immediate causes of death in the frame-work of compartment model methodology, a systematic investigation of statistical procedures is being developed for use in analyzing epidemiological data. Methods developed during the past year provide a methodology of estimating both primary and secondary decrement rates. Results can be extended to give estimates of relative risk of death due to similar or different immediate causes assuming similar or different chronic disease states. The methods can also be used to estimate latency. The results of the investigation of jackknife methods indicate that even the first and particularly higher order jackknife methods do as well as more involved bias reduction techniques such as those using approximate Taylor series representations.

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