ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Fiscal Year 1980

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

United States NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1980 ANNUAL REPORT of program activities

October 1, 1979 through September 30, 1980

CONTENTS

OFFICE OF THE DIRECTOR

The chemical wastes at Love Canal, New York, and the concerns of the Vietnam veterans over exposure to Agent Orange have emphasized the need to identify those chemicals in the environment which may be toxic and to be able to recognize effects of chemicals on human health. The programs of the Institute, particularly those directed toward development of better tests to predict health effects, as well as actual testing programs, will aid in the solutions to these problems.

Construction of the new South Campus facility continues. The completed warehouse and shops buildings were occupied in October. The Utilities Plant is nearing completion. Construction progress on the administrative modules prompts expectation that occupancy can take place soon after the beginning of 1981. The laboratory modules are currently scheduled for completion beginning in 1982.

As a component of the National Toxicology Program, the NIEHS has devoted particular resources to areas essential to the strengthening and coordination of this DHHS program. This year 11.1 million of the Institute's budget was devoted to NTP. Twelve additional people have been hired by the Institute to help administer the growing program.

The Intramural research program of the Institute continues to distinguish itself through outstanding contributions to the data base necessary to cope with environmental influences on the health of individuals and populations. One such study has resulted in a method to identify and detect point mutation in sperm, which when validated can be used as a system to monitor the human population for induction of mutations and to evaluate the risk of the human population to environmental pollutants. It is the first time that a mutation in sperm which is likely to be a point mutation has been detected.

In another laboratory, investigators are using interspecies in vitro fertilization as an indicator of reproductive capacity, and can then determine changes in this capacity following environmental exposure to xenobiotics. Another study has focused on the chronic effects of PBB in rats and mice over a six month period. Preliminary data indicates a remarkably high incidence of hepatic neoplasm in exposed rats as compared to controls, and that chelating agents and caloric restricted diets are for the most part ineffective in reducing body burdens of PBB. A study on the effects of vinyl chloride using three animal species shows an increase of angiosarcomas in rats, as well as hamsters and a relation between age at onset of exposure and increases in the incidence of angiosarcomas.

Laboratory 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.

The importance of chemistry to the fulfillment of the Institute's mission was cited when the Environmental Chemistry Branch was renamed the Laboratory of Environmental Chemistry, under the continued leadership of Dr. James D. McKinney. The Laboratory serves both as a support activity and as an area for basic research in chemistry. The Laboratory has developed a new way to evaluate the toxicity of environmental contaminants by determining the relationship between toxicity and the size, shape and symmetry of chemical molecules. This new approach to screening for toxicity may provide data useful in selecting chemicals for further testing that are most likely related to the cause of cancer, thus saving crucial time and money.

In the Laboratory of Organ Function and Toxicology research investigators in cooperation with the Institute supported C.V. Whitney Marine Laboratory have described the chain of events within the body leading to kidney damage in chronic cadmium exposed individuals. The study describes the uptake of cadmium metallothionein (CdMT) in laboratory animals both for its pathway of toxicity and for its effectiveness as a model system by which to study cadmium toxicity in a specific kidney cell population.

During the year, the laboratories of Animal Genetics and Molecular Genetics functioned independently as newly formed laboratories. Within LMG there are two very different but mutually supportive programs, one examining basic mutational mechanisms at the genetic and chemical levels and the other concentrating on testing large numbers of chemicals by the contract process while developing and optimizing mutagen test systems. In the Laboratory of Animal Genetics a group has been created to study gene structure and organization while population genetics has begun developing molecular techniques for determining genetic variability in populations. Chemical genetics has turned toward analysis of nucleic acid sequences as well as the proteins they encode. These changes and new programs are designed to unify the research efforts of the laboratory in approaching the central questions concerning the nature of genes and what genes represent as targets for environmental mutagens.

In January 1980, the Board of Scientific Counselors reviewed the Laboratory of Animal Genetics and the Laboratory of Molecular Genetics. In June the Board reviewed three other laboratories: Environmental Biology, Organ Function and Toxicology and Reproductive and Developmental Toxicology. These reviews have been structured to provide small group interaction with the laboratory scientists and to utilize increased participation of peer reviewers in addition to the Members of the Board. These reviews are indispensable elements of scientific management and provide critical analysis of program achievement and structure. Two new members of the Board of Scientific Counselors, one in the area of neurobehavioral science and one in cell biology, are now in the process of appointment.

The intramural research program has begun consolidating three related small laboratory units: Pharmacology, Pharmacokinetics, and the Laboratory of Organ Function and Toxicology. This will further research program coherence and scientific management. This reorganization and merger into the Laboratory of Pharmacology will be complete by the beginning of FY-81 and will result in one laboratory which traditionally has an outstanding record of scientific achievement and will offer an improved structure for research planning.

The Inhalation Facility, which had previously been staffed and managed by the Environmental Biology Branch, has been transferred to the Laboratory of Pulmonary Function and Toxicology. This facility, which is in the process of upgrading exposure instrumentation, can be most effectively utilized in the context of the Institute's Pulmonary Toxicology Program. New research leadership in the area of lung pathophysciology is currently being recruited.

During the fiscal year, the National Advisory Environmental Health Sciences Council reviewed 488 applications assigned to the Institute as primary or secondary assignee. This represents an overall decrease of twenty-three applications from Fiscal Year 1979. One hundred and six awards were made; 88 regular research grants, 1 Environmental Health Sciences Center, 4 Research Career Development Awards, 4 Institutional National Research Service Awards, and 9 Individual NRSAs. These new and competing awards plus the non-competing awards brought the 1980 total awards to 423 active grants, a decrease of 37 awards from Fiscal Year 1979.

The Young Environmental Health Scientist (YES) program continues to be an active and viable program. This award mechanism was recently extended to all awarding Bureaus, Institutes and Divisions of the NIH, new guidelines were developed and the program was renamed the New Investigator Research Award (NIRA). The new guidelines provide uniformity for the management and awarding of this support mechanism within all awarding units.

NIEHS is currently supporting 42 NIRA grants in 35 institutions. This represents an increase of 21 awards over Fiscal Year 1979 and is an indication of the acceptance of this program by the scientific community as a mechanism for support of new faculty who have not yet received support through a regular research grant.

Investigators supported by research grants through the Extramural Program are continuing to look into the health effects of formaldehyde and hydrochloric acid. Previous work has shown that a number of volatile, direct acting halogenated organic compounds can cause squamous carcinoma of the nasal cavity. Subsequently, mixtures of HCl and formaldehyde which react readily in the test tube to form bis(chloromethyl)ether, have been shown to cause a high incidence of nasal carcinoma in rats. Current studies are designed to determine whether the substances react in the air or in the mucous layer of the nasal passage to form the carcinogen. In other research at New York University studies are underway to investigate whether certain polycyclic aromatic hydrocarbons (PAHs), benzo(a)pyrene, and 7,12, dimethylbenz(a)anthracene might be implicated in the formation of atherosclerotic plaque. The

research using the chicken as a model is being extended to include the effects of the components of cigarette smoke. Although there is strong epidemiological correlation between cigarette smoking and heart disease, there is little direct evidence that cigarette smoke or any of its components except for carbon monoxide are atherogenic. Since PAHs are found in cigarette smoke and there is a correlation between exposure to PAHs and plaque formation, the chicken model may provide the long-sought test for atherogenicity of single compounds and complex mixtures.

Through its research grant program the Institute is currently supporting a variety of research on TCDD (dioxin) including pharmacologic and toxicity studies at the molecular, membrane and cellular levels; toxicity and metabolic studies in fish, frogs, rats and non-human primates; low-level effects in treated animals; and reproductive effects in rhesus monkeys.

The Office of the Associate Director for Genetics continued to fulfill its role in the genetic toxicology program of the Institute by serving as focal point for international and national activities. On the International scene cooperative agreements with Japan were continued in the area of environmental mutagenesis and carcinogenesis. In the U.S. the Office is involved in the efforts of the EPA Gene-Tox program which is evaluating the status of genetic toxicology assays and looking into appropriate test batteries for mass screening or for specific chemical classes, potency correlations and mutagenicity/carcinogenicity correlations. The Office also expects to publish in the near future the results of an International collaborative study for the evaluation of short-term tests for carcinogenicity which involved 65 investigators from 12 countries and more than two years work. The Office is also continuing its efforts to organize a collaborative research effort designed to foster the development of assay systems capable of detecting chemicals which induce aneuploidy.

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

This office is concerned with the evaluation of human health hazards particularly due to pollution by chemicals. It has become an order of highest priority to detect these toxic chemicals, to understand their potential for disaster, and to help protect the public before any exposure took place.

To make health hazard assessments requires an evaluation of many different parameters. Although there appear to exist no-effect levels for all chemicals, those that tend to accumulate in the biosphere require special consideration of dose/response relationships. Also where synergistic effects can be demonstrated, greater attention needs to be paid to these realistic situations. Human variation in susceptibility, based on genetic factors or disease condition, must also be taken into consideration.

One task of this office is to bring together for evaluation all metabolic mechanisms in mammalian systems which affect toxic chemicals and can alter or prevent the adverse results of exposure to them. It deals with multiple pathways of metabolism and modification of the pathways by nutritional or other environmental factors which might affect the absorption, retention, activation or deactivation, and final elimination of the chemical.

Importance is attached to the problem of overloading of metabolic pathways and creation of other mechanisms of disposal of chemicals, an issue of importance in extrapolation from high to low dose levels. The true impact of DNA repair mechanisms in preventing permanent damage to DNA also needs elucidation.

A subcommittee to the NTP Chemical Nomination and Selection Process Committee was set up at NIEHS under the chairmanship of Dr. Falk with participation of all members of OHHA as well as Drs. Douglas B. Walters and Bruce A. Fowler of the NIEHS Intramural Program and Dr. Dean Smith of the EPA. The activity of this subcommittee was focused primarily on potential health hazard from organometallic complex formation and emission of toxic metals resulting from the synfuel processes, i.e. the gasification of coal. The exploration of potential hazards was not limited to the effluent streams or the final product, but dealt also with the disposal of clinkers and flyash and the mobilization of toxic metals from such deposits.

The subcommittee also concerned itself with problems of testing for genotoxicity of compounds of these elements which are not readily detected by the standard mutagenicity tests.

Because of the importance of the toxicologic potential of exposure of populations to toxic chemicals at the Love Canal dump, the subcommittee assessed the known toxicity data available in the literature for all so-far identified chemicals from that dump compiled by Naomi Jean Bernheim. Subsequently, the

subcommittee reviewed the possibility of interactions between these chemicals in the exposed population.

A search of the literature was carried out on bioassay results on hazards from asbestos replacements considering the urgency of replacement of that type of minerals in a whole variety of industrial uses. The carcinogenic potential of fiberglass was reviewed in depth in this connection, as it would serve as suitable substitute. In certain experiments, fiberglass however could also be shown to be carcinogenic, although the risk appears to be far smaller than from asbestos.

One of the programs of this office is the compilation of data obtained from the literature on human disease conditions for each organ and tissue to be correlated with exposure to chemicals and to establish a potential cause effect relationship by a search for correspondent laboratory data.

This office continued to serve towards the implementation of the Toxic Substances Control Act. Dr. Piver remained as alternate on the Interagency Committee for Selection of Substances for Testing. Dr. 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 HEW Committee to Coordinate Environmental and Related Programs. She also serves on the TIS Task Group for the Laboratory Data Bank, an on-line computer system providing comparative pathological data on laboratory animals of different strains. Besides membership on the Chemical Substances Information Network Subcommittee, she is a member of the Interagency Response to Chemical Concerns Committee.

Collaboration with the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) has continued. Dr. Falk participated on Interagency meetings on the implementation of the WHO's International Programme on Chemical Safety (IPCS) to be activated on a national level, but coordinated by WHO. Dr. Vouk retired from the WHO and joined OHHA as a visiting scientist to initiate the IPCS at NIEHS and to coordinate this activity. He participated in the IPCS meeting April 9-11 held at NIEHS which layed the foundation for the activation of the program.

Dr. Falk remains a member of the International Joint Commission's Committee on the Assessment of Health Effects of Great Lake Water Quality and is involved together with Dr. Damstra in the evaluation of 450 chemicals detected in the Great Lakes for their potential carcinogenic and neurotoxic properties. This information will be combined with other evaluations regarding potential toxicity and lead to in-depth studies on quantitative aspects of these observed contaminations of the Great Lakes. He also attended the fourth meeting of the Scientific Advisory Committee for the United Nations Environment Programme's International Register of Potentially Toxic Chemicals (IRPTC) in Geneva, Switzerland, November 12-16, 1979.

The Subcommittee on Laboratory Chemical Carcinogen Safety Standards of which Dr. Falk was a member has completed its report.

Dr. Posner continued his participation in the Interagency Committee for Stratospheric Ozone Protection (ICSOP) and remains actively involved in the "paraquat spraying of marihuana" problem, assessing health effects of alternatives and assessing the effectiveness of potential marker compounds as part of a DHHS ad hoc committee.

Dr. Falk was a member of the subcommittee to NTP which reviewed questions presented by Proctor and Gamble regarding the mechanism of NTA carcinogenicity. A final report was reviewed by the Executive Committee of NTP and transmitted to Proctor and Gamble.

Dr. Falk became a member of the Chemical Industry Institute of Toxicology (CIIT) scientific advisory panel and remained a consultant to Cornell University on a program dealing with International Analysis of Public Policies Concerning Carcinogens. He also was asked to give a seminar at the University of South Carolina in Columbia on Hazard Assessment regarding Carcinogenic Exposures.

The staff of OHHA continued to collaborate under guidance from CDC in giving constructive criticism on documents prepared by other agencies on the topic of environmental/occupational health hazards. The staff also reviewed grant applications received by USDI on research in the area of water pollution and purification.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20002-08 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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 and apply techniques for technology forecasting and technology assessment for the chemical process industries that would provide guidance in setting research priorities for environmental chemicals for the Toxic Substances Control Act and the National Toxicology Program.

PHS-6040
(Rev. 10-76)

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 coal conversion technologies. In particular, emphasis has been directed towards identifying the process conditions that influence the formation and partitioning of inorganic and organometallic compounds in different components of the coal gasification process. A very thorough analysis of the technology of coal gasification was completed with available information. As a result, recommendations were made to identify specific metallic and organometallic compounds and associate their formation with process conditions and coal characteristics. When the identities of specific chemicals have been verified, the chemicals will become candidate chemicals for the National Toxicology Program.

The proposed cause is to continue and establish relationship between coal properties, process conditions, and formation of metallic compounds. This program will be extended to other coal conversion technologies such as coal liquefaction and direct combustion of coal. The identification of metallic compounds in process effluent streams is also important in contract activities that are concerned with the transport and uptake of metals by plants because the solid wastes from these effluent streams will be disposed of in terrestial landfills. Another ongoing program effort that will benefit from this program on chemicals formed during coal gasification is a program whose purpose is to establish priorities for toxicity evaluation. This program is seeking a more quantitative relationship between chemicals of similar molecular structure and is attempting to establish relationships between chemicals of known toxic properties and those chemicals for which toxicity information is not available.

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 Interagency Testing Committee of TOSCA, and as a member of the NTP Chemical Selection Committee.

PUBLICATIONS

Piver, W. T.: Deactivation and disposal methods for small quantities of experimental chemicals. In Walters, D. B. (Ed.): Safe Handling of Chemical Carcinogens, Mutagens, Teratogens, and Highly Toxic Substances. Ann Arbor, Michigan, Ann Arbor Press, 1980.

Piver, W. T.: Perspectives on Energy. Sciquest 53: 17-21, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20003-07 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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
	Alan G. E. Wilson	Visiting Associate	LPK	NIEHS
	Douglas B. Walters	Tech. Program Mgr.	ECB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
OD, LPK, ECB

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 project uses a variety of techniques for early awareness and attempted reduction of chemical- and physical-agent-mediated health hazards. The areas of usage of vinyl halide-type compounds, methanol, and aminimide-type compounds continues to be monitored for human health-related aspects. A contract is being monitored regarding more accurate determination of the amount of paraquat that comes through marihuana main and side stream smoke under more normal smoking conditions. The safety of potential odorant markers have also been surveyed. Participation in research planning and reporting to Congress with regard to human health aspects of stratospheric ozone reduction continues in association with an interagency group. In collaboration with Dr. Hans L. Falk and others of our group, activity has been heavy in the areas of potential human health hazards in association with coal conversion (gasification initially) and the use of asbestos substitute materials.

PHS-6040
(Rev. 10-76)

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. Most of the reports of human involvement with these compounds remains concerned mainly with the two carbon homologs with one or more halogen atoms. Animal in vivo and in vitro studies are expanding with regard to larger and somewhat more complex structures. I was able to attend a recent symposium on the subject and exchange ideas with investigators in the field.

Methanol: Pressure continues for the use of methanol as an alternative fuel though, in this country, ethanol continues to be the alcohol of choice for gasohol. Methanol can be obtained in the open market and is found in industrial situations so that it could be consumed, inhaled, or through contact with skin lead to poisoning of people. One case of an infant's death was attributed to the use of compresses on the chest where ethanol was mistakingly replaced by methanol. A similar situation had occurred in Argentina where 20 children treated with methanol died while out of 30 infants treated with ethanol only one died. Methanol remains a hazardous chemical.

Paraquat: A contract to determine more accurately how much paraquat comes through marihuana cigarettes in mainstream and sidestream smoke under more normal smoking conditions continues. The hazard of paraquat and the proposed odorant marker, d-limonene dimercaptan have again been reviewed and the conclusion reached that the choice of these two agents is a poor one from a health standpoint. The entry of marihuana into the United States from Mexico is being reduced, but it is being replaced by imports from other countries. Paraquat has been reported to be mutagenic and to react with DNA. More toxic effects in animals have also been reported on exposure to paraquat in conjunction with other chemicals. In September 1979, the decision was made that the U. S. would not continue to fund the spraying of Mexican marihuana with paraquat.

Stratospheric modification: I participated in the planning of research and the preparation of the Biannual Report to Congress with regard to high altitude pollution and its effect on stratospheric ozone, having secondary effects on human health. Others were involved with effects on animal and plant life and the ecosystem in general.

Aminimide compounds: Research occurring on human health-related aspects of these types of compounds declined considerably over the past year. The literature will continue to be followed.

Hazards accompanying coal gasification schemes: The chemistry of the minerals of coal and some of their potential conversion products during gasification was surveyed. An advanced course was attended. This work has been done in collaboration with Dr. Hans L. Falk, Dr. Warren T. Piver, Dr. Terri Damstra, Dr. William Jurgelski, Jr. and Naomi Jean Bernheim of NIEHS.

Asbestos substitutes: An in-depth review of materials that have been used in any of the wide range of asbestos applications was made. These were sorted by chemical and physical types.

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

Other NIEHS and outside activities: Draft documents prepared by several Government agencies were reviewed for their health hazard content. Requests for information are also answered.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas were 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.: Chemical causes of prenatal maldevelopment. In Walters, D. B. (Ed.): The Safe Handling of Chemical Carcinogens, Mutagens and Teratogens: The Chemist's Viewpoint. Ann Arbor, Ann Arbor Science Publishers, Inc. Vol. 1, 365-384, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20004-06 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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
	Jane Ellen Simmons	Biologist	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)

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☐ (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.

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, and cardiovascular 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.: Neurochemical approaches to the detection of neurotoxicity. In Mitchell, C. L. (Ed.): *Target Organ Toxicity*. New York, Raven Press. (In press).

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. (In press).

Rogan, W., Bagniewski, A., and Damstra, T.: Pollutants in breast milk. N. Engl. J. Med. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20007-03 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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. 10-76)

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. As part of this effort, he prepared a review of the acute and chronic human and animal toxicity of metal carbonyls.

2. The Principal Investigator prepared a review on potential interactions among chemicals identified in the Love Canal waste dump. These interactions were found to involve either the potentiation of the toxic (non-carcinogenic) effects of one chemical by another or the enhancement of the progression of latent neoplastic changes (promotion or co-carcinogenesis). Possible interactions in skin, liver, and kidney were discussed.

3. The Principal Investigator participated in a review of interaction tests that could be utilized by NTP in relation to chemical dumps and related situations. He wrote the preface and developed the sections on co-carcinogenesis, carcinogen formation from reaction of non-carcinogenic chemicals, inhibition of DNA repair, chromosome abnormalities, and interactions leading to changes in metabolic profiles and enzyme induction.

4. The Principal Investigator prepared an evaluation of the health effects of fiberglass as part of review of asbestos replacements and their potential health hazards. This review revealed strong evidence that fiberglass is a carcinogen when the fiber size is comparable to asbestos. However, based on the available information, the potential health effects of fiberglass under the conditions of current manufacturing practices would not appear to rule out this material as a substitute for asbestos if data on human exposure levels under the conditions of the various applications were obtained.

At the request of the Director, the Principal Investigator represented the Institute at a Workshop on The Toxicity of Benzene at the Mt. Sinai School of Medicine. The Workshop was arranged in the hope of establishing a continuing non-adversary dialogue between the several industrial and academic/governmental groups concerned with the toxicity of benzene.

The Principal Investigator also attended a conference on Carcinogenic and Mutagenic N-substituted Aryl Compounds sponsored by NIH.

As part of the Proposed Course, a series of papers is being prepared that will provide a detailed review of chemical interactions.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20008-03 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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. 10-76)

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

(1) 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.

(2) 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.

(3) 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).

(4) 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).

(5) 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, Stanford University School of Medicine.

The Principal Investigator was invited to present an evaluation of the marsupial as a laboratory animal by the Institute of Laboratory Animal Resources, National Academy of Sciences. He was also invited to present a seminar on experimental pediatric carcinogenesis and the marsupial as a laboratory animal by the Facultad De Ciencias, The University Nacional Autónoma De México.

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.

PUBLICATIONS

Jurgelski, W. Jr. The Marsupial as a Laboratory Animal. ILAR News, Institute of Laboratory Animal Resources, National Academy of Sciences, 22: 18-21, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20009-02 OHHA

PERIOD COVERED
October 1, 1979, to September 30, 1980

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

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

PI:	Jean Bernheim	Microbiologist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
	Terri Damstra	Biochemist	OHHA	NIEHS
	Warren T. Piver	Chemical Engineer	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	

CHECK 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. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The open literature is consulted for identification of chemical structures that are associated with certain biological or toxicological effects. This information is collected, classified on the basis of 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 by the Eckhardt-Moffett hearing on May 22, 1980.

Chemical/Chemical Interactions: This is a long-term project which has been initiated because of the multiple nature of most chemical exposures to the human population. An open-ended file has been started to collect specific types of interactions. A search of the available mutagenicity tests was made and a summary of the tests most appropriate for testing interactions by NTP was included in an interaction test report to Dr. Rall of February 22, 1980.

DNA Repair Induction: An adaptive response -- an inducible "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.

DNA repair inhibitors: This search of the literature will have to continue for some time as the topic becomes of greater interest to scientists and more data will be published. A distinction must be observed between general protoplasmic poisons and specific inhibitors of specific DNA repair enzymes. This is an important area which is only recently activated.

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.

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.

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 electrically neutral ones. Using the soil perfusion column, neutral organometallic complexes of nickel produced by fungi have been isolated. Work is now in progress to determine the chemical characteristics of these complexes. The identification of the chemical constituents of the complex will indicate probable processes by which soil microbes modify the chemical form of the metal, and the role of these processes in making metals more available for plant uptake. Similar studies are being carried out for cadmium, chromium, and thallium.

In kinetic studies with metal uptake by plants, studies are in progress to determine physiological processes that are important in transporting the metal across the root membrane and translocating it to different parts of the plant. These studies are continuing for nickel.

The biochemical studies which tie together the microbial studies and the uptake kinetic studies, are continuing to improve the selectivity, sensitivity, and efficiency of separation and identification procedures. Continued research is required to find better solvents, buffers, and chromatographic supports.

A stochastic model is being developed to help identify rate limiting processes in the soil-plant system. From this information, research programs will be initiated to describe underlying mechanisms involved in these rate controlling processes that will include the action of the metal itself and its interaction with other constituents of this system.

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.

PUBLICATIONS

Drucker, H., Garland, T. R., and Wildung, R. E.: Role of soil microbiota in chromium modification. In Proceedings of Intra-Science Symposium on Trace

Metals in Health and Disease. Santa Monica, California, Intra-Science Research Foundation, November 29-December 1, 1978.

Wildung, R. E.: Significance of environmental pathways in pollutant toxicology. In Proceedings of Intra-Science Symposium on Trace Metals in Health and Disease. Santa Monica, California, Science Research Foundation, November 29-December 1, 1978.

Wildung, R. E., Garland, T. R., and Drucker, H.: Nickel complexes with microbial metabolites -- Mobility and speciation in soils. In Jenne, E. A. (Ed.): Chemical Modeling in Aqueous Systems. ACS Symposium Series 93, 1978.

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64110
(NIH-NIEHS-78-2153)

TITLE: An Appraisal of Environmental Exposure to Heavy Metals

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, 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: $77,029

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this program are: (1) identify sources and routes of human exposure to heavy metals as a result of the various steps and industrial operations involved in the commercialization, utilization, and disposal of metals and their compounds; (2) examine and present available information on the toxicology of the metal and its compounds, and to identify analytical methods and their limitations for quantitative measurement of metals in environmental and biological media; and (3) forecast trends in metal production that may produce increased environmental entry and exposure to metals.

METHODS EMPLOYED: The methods used to perform these tasks are the use of literature reviews and expert opinions from industry in order to compile data on sources of release and exposure to metals and their compounds. Special interest is placed on determination of the physical-chemical properties of the metal that influence how the metal makes contact with man. The information is put together in a report on each metal of specific interest.

MAJOR FINDINGS AND PROPOSED COURSE: Within the past year, a report on cobalt has been produced. These reports have a special significance because they attempt to define the chemical state of the metal in the environment as a result of its release due to intended use or as a contaminant in air and water. These reports have been published in book form and are available to a wide readership. This program was terminated after completion of the cobalt report.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals and their inorganic and organometallic derivatives and complexes. The information determines gaps in understanding of the health effects of the metallic compounds in question and the type of research to be undertaken. In addition, these documents have been sent to biomedical researchers who are with Federal and academic programs, and to program directors in foreign countries. Comments

received from these people have indicated that these reports are timely and provide useful information for planning research programs.

PUBLICATIONS

Carson, B. L., and Smith, I. C.: Cobalt: An appraisal of environmental exposure. Contract No. NO1-ES-8-2153, December 1979.

PROGRAM PLANNING AND EVALUATION

OFFICE OF PROGRAM PLANNING AND EVALUATION
Summary Statement

Introduction

After three years of operation it seems appropriate for OPPE to look back on developments in the field of environmental health and forward to what those developments portend for NIEHS.

Twenty years ago the Final Report of the Study Group on Mission and Organization of the Public Health Service was published. This report, which called for a major reorganization of the Public Health Service, looked forward to the last decades of the century and anticipated that the problems of the environment would be among the most serious health issues facing the Public Health Service and the Nation.

In its section on environmental health, the report stated:

> "One of the sharpest dilemmas confronting the American people is how to create healthful environments in a highly industrialized, urbanized society and at the same time retain the benefits of modern technology. Natural and artificial components of the physical environment are essential to life and health. Technologic developments of the contemporary period have added enormously to the healthfulness and convenience of daily living. Yet these benefits have not been won without heavy hidden costs in terms of environmental hazards and of economic effects.
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> * * *
>
> "There is relatively little definitive knowledge concerning many factors in the contemporary environment and their health effects. Many chemical substances and physical devices, now familiar components of the daily environment, were unknown ten years ago or affected very small proportions of the population in limited occupational situations. Lack of knowledge is the principal handicap under which governmental agencies at all levels struggle in their efforts to provide adequate protection of physical resources and human health.
>
> "There are many gaps and inadequacies in existing knowledge and methods which deter effective action. Methods of measurement for many environmental factors are inadequate and incomplete. Many methods of control are antiquated and only partially effective. However, the great unknown is the biological and health effects of these substances which impinge on man from many sources, typically in intermittent doses, and over long periods of time. Fulfillment of the responsibilities of the Public Health Service in environmental health will require intensive study of these aspects in the laboratory, in the clinic, and in the field.

"In the next ten years, it will be necessary to augment the Nation's environmental research effort so that these critical gaps may be filled. The vast improvements in basic and applied research techniques within the past ten years offer hope that their intensive application will provide the knowledge essential for rapid advances in the solution of environmental health problems."

It is now clear that the members of the Study Group had a clear vision of these emerging problems, although no clear sense of the extent to which these problems transcended national and geographical barriers. It can now be said that there are no "trade barriers" to pollution.

It is equally true that the PHS did not then have the institutional will to respond to those challenges. Further, this holistic view of the environment was swept over by the environmental protection movement of the late sixties and early seventies, thus causing us to lose sight of the fact that in the final analysis it is man's health and his ultimate fate which is inextricably intertwined with his environment; that our ecology includes us as well as plants and animals. Although reasonable people do not question the long-term importance of a healthy environment, they have and do fail to recognize that man is the ultimate beneficiary of our actions to improve and strengthen the health of our environment.

In addition we have come late to the understanding of the linkage between today's health problems and other problems which are not specifically health in nature. Further, and critically important is the rate at which we are beginning to recognize environmental issues as having a health focus, and how often that recognition preceeds our scientific understanding of the issue or technological capacity to prevent potential or even further harm to health.

But recently, as we said in last year's summary, this field has been greatly impacted by three developments:

1. The growing public recognition that environment and health and other societal concerns are interrelated.

2. The increasing need for both the Congress and the public to understand environmental health issues that bear on problems which do not have health as their central focus.

3. Greatly increasing Executive Branch activity in this area, which requires that the Institute be able to focus on the interrelationships between the science, legislative, regulatory, and public policy components of environmental health issues.

But increasingly manifest is the current inability of either the Executive or the Legislative to conceptualize the problems of the environment and environmental health as either a single issue or a broad set of interrelated issues. The current proclivity of our national institutions to deal with problems as "single issues" causes us to lose sight of their interrelatedness, and leads us to view toxic waste dump problems as somehow separate

from problems of air pollution and water quality, when in fact we are learning that most of our environmental hazards seem to settle into our water for resolution. Our difficulty in achieving a unitary philosophy and approach to problems of the environment leads us to fail to recognize that the quality and adequacy of our water and man's well-being seem to be parts of the same larger issue.

It is against this backdrop of problem identification, and program development that the NIEHS and its programs have been established, evolved, and nurtured. Further, the interrelatedness of problems of the environment has caused the NIEHS to look outward at other government as well as non-government activities to develop effective means of collaboration and program integration. Among these other organizations are:

1. Other NIH components.

2. The National Toxicology Program (NTP) which includes the following non-HEW agencies:

    . Environmental Protection Agency (EPA)
    . Consumer Product Safety Commission (CPSC)
    . Occupational Safety and Health Administration (OSHA)

3. Committee to Coordinate Environmental and Related Programs (CCERP)

4. The Office of Science and Technology Policy (OSTP)

These coordinating and cooperative relationships require that the NIEHS develop and maintain indepth knowledge and understanding of these other organizations. As the Institute's formal planning arm, it is necessary that OPPE itself carry out its activities with a clear sense of the many government programs related to environmental health.

The activities of OPPE over the last year covered its entire range of responsibilities. In addition the OPPE was called upon to review a variety of program documents, prepared by other agencies, covering a variety of science and public policy issues.

These activities are summarized below in two ways:

1. Areas of substantive program activity.

2. Areas of functional activity.

Substantive Program Activities

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

Asbestos: (Ms. Hoffman)

As in 1979, asbestos and its adverse health effects continued to occupy more staff energy than any other single problem. A variety of asbestos-related activities were carried out in support of the Director. Among these was the drafting of a legislative proposal as an administration alternative to legislation being considered in the Congress. The House-passed legislation provided the basis for the bill enacted into law and known as the Asbestos School Hazard, Detection, and Control Act of 1980 (PL 96-270). OPPE also drafted the Director's testimony on this subject to deliver before the Subcommittee on Education, Arts and Humanities of the Senate Committee on Labor and Human Resources.

Out of this legislative activity grew the recognition that many of those in the educational community who were effected by the problem of asbestos in schools had only slight understanding of the health issues involved. Accordingly, in Fall 1979, OPPE suggested and arranged a meeting between asbestos experts and school educators about the possible health problems associated with asbestos in their schools. A few dozen educational groups attended, and OPPE sent out the transcript of the meeting to a number of others who requested it.

Toxic Chemical Waste Dumps: (Ms. Hudson; Mr. Kingman; Ms. Stopinski)

The generic problem of toxic chemical waste dumps has required the time of a number of key OD staff; OPPE activities in this area were in support of Institute scientific staff. Among the OPPE activities was preparation of a memorandum which evaluated the authorities the government has under TSCA, RCRA, and FEMA to provide technical, financial, and research assistance, and recommended legislative and administrative options for dealing with such problems. OPPE staff also participated in preparing and editing some of the many briefing memoranda which were developed on the problem of toxic dumps.

In addition, OPPE prepared analyses which compared 13 proposals for clean-up and compensation as a result of toxic waste dumping. These memoranda were incorporated into other OPPE staff efforts and were provided the Director and Deputy Director for their background on these emerging legislative issues.

Dioxin/Agent Orange: (Ms. Hudson; Ms. Stopinski)

The Director and Deputy Director, NTP, have significant involvement in the Departments' research on and evaluation of the health hazards associated with human exposures to dioxins and Agent Orange. In support of this effort, OPPE provided analyses of pending legislation, tracked legislative developments, and developed a chronology on dioxin as background for OPPE efforts in this area. In addition, the office provided a current awareness service for the Deputy Director, NTP, who is responsible for the Scientific Panel of the Interagency Work Group on the Health Effects of Dioxins.

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 HHS and the 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 has contracted with the Institute of Medicine for the purpose of developing a plan for the required study. The IOM committee, which is composed of individuals representing a wide array of disciplines, has been meeting throughout the year.

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

The task assigned the committee is extraordinarily complex, and currently impossible given the state of our knowledge regarding environmental factors and their precise impact on specific diseases. However, we believe that for the purpose of trying to develop rational public policy it is possible to develop better estimates of the cost of environmental disease than currently exist.

The committee is currently scheduled to complete its work by late October. Given current budget stringencies, it is unlikely that the full study will be begun prior to Fiscal Year 1983. OPPE continues to believe that the results of such an ongoing study would be of great value in the public policy debates associated with attempting to answer the fundamental question of: "What are the savings, at the margin, in both dollar and human terms of efforts to restrict our exposures to toxic environmental agents?"

National Toxicology Program: (Ms. Hudson; Mr. Kingman)

As the National Toxicology Program has become a functioning reality, and begun to develop its own staff resources, there has been less need for a significant investment of OPPE staff resources in this program.

However, OPPE continues to be called upon to take staff responsibility for specific and frequently rush activities relating to NTP. An example of this was the NTP's response to the President's Office of Management and Budget (OMB) request that the Secretary, HHS, develop program plans for the Department's National Toxicology Program (NTP). Based upon this request, OPPE worked with the NTP agencies (NCI, FDA/NCTR, CDC/NIOSH, CPSC, EPA, and NIEHS) in developing the document, "National Toxicology Program - Program Plans for FY 1981." This plan provided an assessment of NTP's efforts and laid out a comprehensive multi-agency program for action through FY 1981. It gave detailed descriptions of the NTP goals, organization, principal elements, activities, progress, constraints, needed resources, guiding philosophy, and evaluation. Finally, the plan attempted to document the NTP's role in going beyond individual agency missions to attack national problems.

As part of the Secretary's continuing effort to establish a Department-wide process of health research planning, the OPPE also continued its work aimed at the development and acceptance of a five-year plan for NTP. The Secretary's effort is intended to guide the Department in its allocation of limited resources, and has two major goals: (1) to develop a common point of reference against which HHS' health research activities can be examined, and (2) to develop proposals for long-range budgeting of programs of special importance. The NTP is one of these special programs; and its five-year plan is addressed to obtaining a long-range commitment of support for the program, one of whose inherent characteristics is the multi-year duration of existing animal test procedures. The NTP Five-Year Research Plan developed by OPPE as an expression of the goals of the NTP agencies extends the comprehensive multi-agency program for action developed for FY 1981 through FY 1985; and it specifies detailed policies and goals for the NTP.

OPPE believes that a "rolling" five-year planning system is a necessary ingredient for the development of continuing broad-scale acceptance and support of the NTP.

Health Promotion and Disease Prevention: (Ms. Hudson)

Throughout the past year the OPPE has been called upon to participate in and contribute to the ASH effort to develop a PHS-wide program in health promotion and disease prevention. This effort, which was difficult to develop and carry out on a broad scale, has required continuing OPPE input for and review of ASH developed documents.

Risk Assessment: (Ms. Hoffman)

In mid-year the OPPE began to develop knowledge of risk assessment as an activity and its relationship to the development of effective public policy. It is hoped that this effort will be useful to the OPPE as it proceeds with its program planning and legislative support activities.

As part of this effort OPPE prepared a talk which was submitted as part of the proceedings on a New York Academy of Sciences Conference on Risk Assessment.

Program Planning Activities
(Mr. Kingman)

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, as discussed earlier, the OPPE has been involved in a variety of long-term, continuing and one-time planning activities.

This complex of both structured and ad hoc activities has set the parameters within which OPPE must work to develop its program planning activities for the years ahead. It is believed that over-emphasis on structure and order can result in plans which are both rigid and irrelevant to the world in which NIEHS must function. By the same token increased emphasis in this area can result in a planning system which is flexible enough to accommodate to the Institute's changing needs and, by careful planning, can reduce the continuing burden on the program staff who must contribute to the Institute planning process and its resulting documents.

In the coming year the OPPE will be working to develop means for strengthening the Institute's planning process.

Program Evaluation Activities
(Ms. Hudson)

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

The evaluation planning cycle for FY 1981 got underway during the Winter of 1980 when OPPE developed the NIEHS FY 1981 Evaluation Plan. Subsequently OPPE met with NIH, ASH, and OS program evaluation staff for a review of NIEHS' plans for the upcoming year and the progress of NIEHS' evaluation project.

The evaluation project reviewed for the HHS Assistant Secretary for Planning and Evaluation and the PHS Deputy Assistant Secretary for Health Planning and Evaluation involves the evaluation of the NIEHS environmental toxicology training program. In its second year, this project is being carried out by a contractor under the direction of Extramural Programs staff. It is structured to evaluate the impact of the NIEHS toxicology training program and to serve as the basis for decisions on program direction and funding levels by the NIEHS Director and program staff.

One of the expected outcomes of this project is that, by monitoring the manpower situation, NIEHS should be able to identify changes that are taking place in requirements for trained toxicologists, permitting it to take appropriate actions to revise its training program where needed. Thus, the completed project will enable NIEHS to carry out effective program planning and budgetary decision-making for its environmental toxicology training program. Another expected result is that improved information on the manpower situation should contribute significantly to improved national policymaking in areas related to broad manpower issues, not only in the environmental toxicology field.

When completed, OPPE hopes to use this evaluation project as a model for the establishment of baseline data which can assist in evaluations of the Institute training programs in environmental epidemiology and biostatistics, environmental mutagenesis, and veterinary pathology. OPPE hopes to begin such an evaluation of the environmental epidemiology and biostatistics training program in 1982.

In looking to the years ahead, OPPE hopes to better integrate the Institute's program planning and evaluation activities to improve their utility to policymakers and program leaders.

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; and 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. The major OPPE legislative activities on specific topics have been discussed previously. What is discussed here is the baseline legislative effort which supports specific Institute legislative analysis activities. This service is designed to keep the Institute 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 1980 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 resource conservation and recovery--toxic waste dumps, dioxin, asbestos, and biomedical research); to tracking an array of some 375 bills through the Congressional 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, HHS 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 HHS officials; and developed briefing information for Congressional leaders and staff.

To assist in keeping the Director's staff abreast of the latest legislative issues, a list of upcoming hearings was prepared and sent to the OD staff weekly when Congress was in session.

OPPE also maintained its working relationships with key legislative information and support services such as the Congressional Research Service staff.

Administrative Considerations

There are a variety of administrative considerations which impact on the work of OPPE. Foremost among them is the current severe stricture in personnel staffing. This is particularly difficult because a relatively small amount of the workload is determined by OPPE. In order to be truly 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. 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, the NIH Institute Planning Officers will soon begin a study of the NIH program analysis positions in an effort to propose an appropriate standard to the Office of Personnel Management. In any event, 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.

Based upon the rate at which OPPE is increasing its library and research file holdings, it is clear that in the years ahead OPPE will have to develop some new and more space conscious approach to handling its research files.

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. This, combined with the Mag II typewriter's ability to communicate rapidly across telephone lines, has greatly increased the flexibility and productivity of OPPE.

The increasing knowledge and productivity of the support staff has made it possible for the OPPE Director to delegate responsibility for much of the normal, although critically important, office administrative responsibilities.

Summary

The three years since the activation of OPPE have seen extraordinary developments in environmental health and in the related programs designed to aid in the protection of public health. The array of activities in which NIEHS has been involved has been impressive. OPPE has been able to contribute to the development of new institutional arrangements and programs to improve the government's performance in this area. It has been an exciting and fulfilling, although occasionally frustrating, three years. Much remains to be done. Much lies ahead.

GENETICS

During FY1980 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) Training, (4) Committees, (5) Collaborative Studies, (6) Collaborative Research Programs.

International Programs

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 guidelines for the Joint Panel were revised in a meeting with the Japanese Panel during 1978. The guidelines were revised to shift the major emphasis of the activities from the development of short-term assays and testing programs to human population monitoring and epidemiology. In line with this shift in emphasis, the membership of the U.S. Panel was changed during FY80. Drs. D. Brusick and V. Dunkel, experts in the field of short-term testing, were replaced with Drs. I. Selikoff and J. Mulvihill who work in the areas of human health and population studies.

The 9th Joint Meeting of the Panels, held in Karuizawa, Japan, September 4-6, 1980, dealt with the topic "Mutagens and Carcinogens in the Diet and Digestive Tract". The program was divided into five sessions dealing with mutagens and carcinogens in (1) raw foods, (2) cooking and processing, (3) the digestive tract, (4) human and animal waste and, (5) the modulation of mutagenic and carcinogenic activity by dietary factors.

US-USSR

In the October 1979 issue of the Proceedings of the National Academy of Science (USA), Dr. N. P. Dubinin and colleagues published a paper which reported a 1000-fold higher frequency of electrophoretic variants among congenitally malformed children than in normal children in Moscow. Under the auspices of the US-USSR Agreement in Environmental Health, a mechanism has been developed to allow collaboration between Professor J. V. Neel at the University of Michigan in Ann Arbor with Dubinin's group at the Institute of General Genetics in Moscow. In this collaborative program, it is proposed to evaluate the blood samples from the Soviet study in Ann Arbor to confirm and extend their observations as well as to try to identify a comparable population of congenitally malformed children in Ann Arbor and environs to independently confirm the USSR scientists claims.

ICPEMC

During FY80 the Associate Director for Genetics attended two meetings of the Executive Board and Commission of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC). Due to the failure of ICPEMC to obtain contract funds to support Committee activities, an emergency Executive Committee meeting was held in Basel, Switzerland on February 23-26, 1980, to develop a revised schedule of Commission, Committee and Task Group activities during CY1980. The Executive Committee and Commission met again in Lausanne from September 27-October 3, 1980, to review the work of the standing Committees and papers in preparation by various Task Groups and to prepare program plans for CY1981.

National Programs

EPA Gene-Tox Program - Both the ADG and the Assistant to the ADG are members of the Steering Committee for this program which was initiated in FY78. Most of the 25 workgroups of experts which were evaluating the status of genetic toxicology assays have completed their evaluation of information available in the literature and have reports written in various stages of completion. Two of the reports (E. coli WP2 and Mouse Heritable Translocation) have completed the review process and are now in press at Mutation Research. The Gene-Tox Assessment Panel will conduct the second phase of the Gene-Tox Program by using the workgroup reports to answer broader questions involving appropriate test batteries for mass screening or for specific chemical classes, potency correlations, and mutagenicity/carcinogenicity correlations. The ADG is involved in several of the Assessment Panel's efforts.

Aneuploidy - Manuscripts from the NIEHS sponsored Workshop on Systems to Detect Induction of Aneuploidy by Environmental Mutagens (Nov. 1978) were published in the August 1979 issue of Environmental Health Perspectives. The publication has received widespread attention both in this country and abroad. In line with increasing concern for the significance of this endpoint, the National Toxicology Program will soon award contracts for the development of assays for induced aneuploidy in both yeast and Drosophila.

The OADG has surveyed a number of laboratories conducting research in this area. Information obtained in that survey is now being used to plan an international collaborative study designed to foster the development of aneuploidy assays in a range of eukaryotic organisms from fungi to rodents.

DNA Repair - A book entitled "DNA Repair and Mutagenesis in Eukaryotes" will result from the NIEHS sponsored conference of the same title (June 1979). It is presently in press and should be available in September, 1980. This volume will present the status of research directed at

understanding the role played by repair systems in the mutation induction process in eukaryotic organisms. Knowledge of this role is then related to effects on the qualitative and quantitative risks posed to humans by agents capable of reacting with DNA.

Pollen Workshop - A meeting on "Pollen Systems to Detect Biological Activity of Environmental Pollutants", cosponsored by EPA and NIEHS, was held in Knoxville, TN on May 5-8, 1980. The purpose of this meeting was to (1) review the status of assays for mutagenic and other toxic effects utilizing pollen as the "test object" and (2) determine the extent to which assays have been applied to in situ monitoring for toxic pollutants. Manuscripts from the meeting have been submitted to Environmental Health Perspectives for publication.

Training

The ADG participated as a speaker in the Third Training Workshop organized by the Environmental Mutagen Society in Colorado Springs, CO on February 4-6, 1980. This course was designed to focus on the development of testing programs and the evaluation of test data as it relates to specific industrial and environmental situations.

In addition the Assistant to the ADG is a member of the Environmental Mutagen Society Training Committee which is responsible for developing programs for EMS sponsored training courses.

Committees

The ADG continues to chair the DHHS/CCERP Subcommittee on Environmental Mutagenesis. In about 8 meetings per year, issues of current interest are discussed and speakers are invited to address scientific topics of importance to government agencies concerned with genetic toxicology. Major topics covered during the past year include: comparative mutagenicity of nitrosamines, molecular dosimetry and its application to risk assessment, testing schemes proposed for the evaluation of mutagens and carcinogens, comutagenicity of chemicals, genetic toxicology at the Food and Drug Administration, research programs at the American Health Foundation, and results of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity.

Collaborative Studies

The testing of chemicals under study in the International Program for the Evaluation of Short-Term Tests for Carcinogenicity was completed in August 1979 and the current fiscal year began with a meeting of all participants in that Program (October 17-22, 1979). The meeting was divided into two parts. During the first, investigators using similar assays met in groups to review protocols, data, and individual conclusions. Results of these deliberations were then condensed into group reports.

In the second part, groups were formed to evaluate the overall activity of test chemicals, each group considering all results on seven of the 42 chemicals under study. These groups also prepared reports of their findings. These two sets of group reports along with the individual investigators reports and overview chapters written by Coordinating Committee members will comprise a book containing complete program results and conclusions.

The OADG provided the focus and major coordination function for this program which involved 65 investigators from 12 countries and more than two years work. Having completed the initial program and compiled the book chapters, this office is now attempting to organize and coordinate follow up work which is needed in order to recognize the full potential of this major international effort. Specifically, there were several assays which could not be adequately evaluated because either too few chemicals were tested or data was needed from a larger number of laboratories.

Intense, widespread interest in the outcome of this Program prompted the Coordinating Committee to schedule an early release of preliminary results and conclusions through two public meetings. The first was held at Masur Auditorium at NIH on December 3, 1979. It was attended by approximately 250 people who each recived a summary prior to the meeting and a full transcript following. On December 6, the same basic meeting was held at the Ciba Foundation in London to inform the European press and scientific community of the outcome.

At present, chapters for the book which will contain the complete results are with the Elsevier/North-Holland Publishing Company. A publication date September or October of 1980 is expected.

As discussed under National Programs, this office is continuing its efforts to organize a collaborative research effort designed to foster the development of assay systems capable of detecting chemicals which induce aneuploidy. Both short-term assays for screening large numbers of chemicals and whole animal assays for detecting transmissable germ cell effects as a basis for risk estimation will be included in the study. Preliminary approval of funding has been received and the proposal is now progressing to the concept review stage.

Collaborative Research Programs

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 work is being performed with the collaboration of Illinois State University, Normal, Illinois. Studies on chemical carcinogens are designed to determine whether those chemicals that are carcinogens produce some characteristic type of genetic damage. In addition, various base-line chemical mutagens which produce characteristic types of lesions (base-pair transitions, frameshift mutations, etc.) are

being studied to determine whether similar types of lesions are produced in both eukaryotic and prokaryotic organisms.

In addition, these studies can be used for risk-estimation since they compare the sensitivities of normal strains (wild-type) and repair-deficient strains (excision-repair deficient) with regard to the production of genetic damage after exposure to chemical carcinogens and mutagens. Current data show that in Neurospora these mutagens produce the expected lesions in point mutations at the molecular level, but unexpectedly, they also produce multilocus deletions. In some cases these latter lesions occur at even higher frequencies in the excision-repair deficient heterokaryon. These studies show that the mechanism of mutation-induction may be different in eukaryotic organisms than in prokaryotes. The marked qualitative and quantitative differences between mutation-induction between wild-type (+/+) and excision-repair deficient (xp/xp) heterokaryons make it desirable to look at heterokaryons which are heterozygous (+/xp) since in the human population such individuals would occur at much higher frequencies than (xp/xp) individuals. Experiments to compare the response in heterozygous strains (+/xp) to homozygous strains (+/+ and xp/xp) were started during this past year.

In another area, a test system that has been developed in Neurospora crassa to detect aneuploidy, is being used to screen chemicals for genetic activity. At the workshop on "Test Systems to Detect Aneuploidy" the summary of chemicals which were found to be positive in this system was used to develop a collaborative study to test their effects in other (higher) eukaryotic organisms. These latter studies will serve to determine the general utility of the Neurospora system as a pre-screen for this class of genetic damage in eukaryotic organisms. In addition, new chemicals of interest because of their response or lack of response in other assay systems (eg Ames test with Salmonella) will also be tested for nondisjunction.

From June 1972 to September 79 research was conducted in collaboration with Brookhaven National Laboratory to develop a Mobile Monitoring Vehicle (MMV) with which the mutagenicity of air pollutants could be assessed using the Tradescantia stamen hair assay. The MMV was developed to the stage of application and was operated successfully in a number of pilot studies throughout the U.S. Having supported the development and trial application of the MMV, support was turned over to the EPA which is now using it in air pollution monitoring efforts.

Public Lectures

F. J. de Serres

1. "Role of Genetic Heterogeneity in the Human Population on the Genetic Effects of Radiation", presented at the NIH Science Writers Seminar, September 1979, Washington, DC

2. "Review of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at the Executive Committee Meeting of the National Toxicology Program, November 1979, Washington, DC

3. "Objectives of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at a Public Information Meeting, December 3, 1979, Bethesda, MD

4. "Objectives of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at a Public Information Meeting, December 7, 1979, London, England

5. "Evaluation of the Genetic Effects of Environmental Chemical Mutagens in Neurospora", presented at the North Carolina State University, January, 1980, Raleigh, NC

6. "International Program for the Evaluation of Short-term Tests for Carcinogenicity", presented at the 2nd Annual Environmental Mutagen Society Workshop, February 1980, Colorado Springs, CO

7. "International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at the Workshop Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, March 1980, Williamsburg, VA

8. "Results of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at the 11th Annual Environmental Mutagen Society Meeting, March 1980, Nashville, TN

9. "Evaluation of Short-Term Tests for Carcinogenicity", presented at the American Cancer Society Science Writers Seminar, March 1980, Daytona Beach, FL

10. "Results of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at the Workshop The Predictive Value of in vitro Short-Term Screening Tests in the Evaluation of Carcinogenicity, April, 1980, The Netherlands

11. "Short-Term Tests for Carcinogenicity", presented at Illinois State University, April, 1980, Normal, IL.

12. "Review of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at the Open Meeting of the National Toxicology Program, June 1980, Washington, DC

Michael D. Shelby

1. "Assay Systems/Protocols of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at a Public Information Meeting, December 3, 1979, Bethesda, MD

2. "Assay Systems/Protocols of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at a Public Information Meeting, December 7, 1979, London, England

3. "Results of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity", presented at Colloquium on Quality Assurance of Toxicological Data, December, 1979, Luxembourg

INTERAGENCY PROGRAMS

OFFICE OF ASSOCIATE DIRECTOR FOR INTERAGENCY PROGRAMS
Summary Statement

The Associate Director for Interagency Programs is responsible for the following 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.

1980 was the eighth 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. A number of agencies participate in this agreement, which 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. Effort in this area has been focused on the mutagenic potential of environmental contaminants, the toxic effects of heavy metals in the environment, the toxicity of oil shale products and by-products, neuroendocrine effects, and the effects of pollution in the marine environment.

US-China Cooperation

Cooperation between the United States and People's Republic of China has begun under the US-China Cooperative Health Agreement. Initially, two areas of cooperative activities are being explored under the occupational and environmental health part of the Agreement. The first area is concerned with pesticide standards and usage in the respective countries and the second involves a comparison of short-term, rapid test systems used to predict mutagens, carcinogens, and teratogens. The Chinese Coordinator, Professor Yang Mingding, Deputy Director, Research Institute of Preventive Medicine, Shanghai First Medical College, visited the U.S. from March 18 - June 4, 1980 to initiate discussions on the development of a joint program.

Energy-Related Research

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

During the summer and fall of 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 November 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. As a result of the work for this task force, NIEHS was assigned the responsibility, and given additional funds to develop research programs to address a number of important energy-related questions. 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. During the period 1975-1980, NIEHS initiated a number of new projects focused on problems in these areas.

In January 1976, NIEHS co-sponsored, along with NIOSH, EPA, and DOE, a retreat for 150 scientists working on energy-related health problems. In May 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 initiated a series of jointly sponsored scientific workshops for the purpose of obtaining an up-to-date identification of the health and environmental problems associated with each energy technology and the research needs required to address those problems. In April 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 in December, 1977. 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 1976-1977, 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 June, 1978 and May, 1979, and July, 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 $727.5 million for environmental health research during FY 1981.

As a service to the National Toxicology Program, the Office of Interagency Programs prepares annually a Review of Current DHHS Research Related to Toxicology. This review is required as a part of the annual plan for the National Toxicology Program. This review surveys agencies of the Public Health Service for information on programs in basic toxicology research, toxicology testing, and toxicology method development. Nineteen PHS agencies reported funding for toxicology-related research amounting to approximately $262 million in FY 1980. The review also indentifies the chemical compounds under test for a variety of biological endpoints and toxicology test methods currently under development.

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH
Summary Statement

The Research Services Branch plans and coordinates design and construction of new facilities for the Institute as well as operates and maintains existing facilities. It also provides a variety of research support functions such as laboratory instrumentation fabrication and repair services, arts, graphics, and photography.

Alterations of existing facilities have lagged behind demand, principally due to several major alterations projects that have placed a high demand on manpower resources. The new South Campus facility, currently under construction, is placing greater demands on all personnel within the Research Services Branch as buildings are completed and occupied in the support services area.

NIEHS was allowed beneficial occupancy of the Shops Building and Warehouse on the South Campus in October 1979. RSB management staff moved into the Shops Building on October 5. The Power Plant will be completed and RSB will occupy and operate this facility in late summer.

Administration: Two Sections were established within the Research Services Branch; the Engineering Design Section, headed by Mr. Nathan De Witt, and the Facilities Engineering Section, headed by Mr. Warren Jones. Mr. De Witt recently resigned his position and a search is now underway for a successor. An Administrative Officer position was created to assist the Branch Chief in management of Branch resources and provide administrative and logistical support to all Branch employees. Ms. Elizabeth Ford was appointed to this position.

A management survey of staffing and organization of the facilities engineering functions of RSB was recently completed. The survey reviewed the organizational structure and staffing plans and requirements for the Branch as it expands to operate and maintain the South Campus facilities and equipment. There is a critical need to complete the organizational structure and staffing requirements so RSB can accept the responsibility of operation and maintenance of the South Campus facilities as they are completed.

Facilities: NIEHS currently utilizes 197,000 square feet of leased space, 13,200 square feet of temporary office space in trailer units located on the South Campus, and 56,900 square feet in the support area of the South Campus. The principal interim site provides 154,000 square feet in a complex of 19 buildings. The Institute has a staff housing capability in excess of 500 permanent full-time employees. Facilities operation, maintenance, and operations are carried out by the facilities engineering staff. One of its major activities has been renovating laboratory and office space to meet specific program needs.

New NIEHS laboratory, administrative and support facilities are being constructed on a 509 acre tract of land in the Research Triangle Park that is in close proximity to the current 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 begun in April 1977. The Shops Building and Warehouse have been completed, accepted by NIEHS and occupied. The Power Plant is essentially complete and will be available for beneficial occupancy in late summer. Administrative Modules A and B of the Administration/Laboratory Building are scheduled for completion and occupancy in late fall. Construction delays have resulted in a revised completion date of early 1981 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 approximatley 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 8097 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 8800 sq. ft. off site)	32,590	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	146,071	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	197,011	452,273

Engineering Design: This group provides the architectural and engineering support required for planning new NIEHS facilities, improvements, major repairs, and alterations through in-house design, direct contact with A/E firms, or consultation, liaison and review functions for projects contracted and administered by OES, 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.

A continuing search is being made for qualified engineers to fill one electrical engineering vacancy. Numerous needed alterations, renovations, and repair projects are being held up because of lack of personnel to accomplish the in-house design. An engineering consultant has been reappointed to provide design expertise in mechanical systems and ventilation. He will continue to give attention to many critical ventilation problems throughout the Institute. In December 1979, an Architectural/Engineering Services contract was awarded to provide design services on some of our major projects.

During the year, major activities have been the administration and inspection of the Building 7 renovations; award of contracts for installation of trailers on the permanent site; renovation of machine shop in Building 10; and coordination for the South Campus construction.

Facilities Engineering: This group provides engineering, craft and laboring services necessary for operating and maintaining NIEHS buildings, grounds, utility plant, systems, and related equipment; and contributes engineering evaluation of operational and maintenance aspects of new construction.

The Shops Unit maintained a heavy work load this year. Six temporary journeymen craftsmen were employed to reduce response time on work requests. Even then, many projects were delayed three to six months. Contract documents were prepared for a general construction services contract to perform some of the alterations and renovations work. However, no interest was shown by local contractors and no bids were received. Major activities have been Building 7 renovations, trailer installation on the permanent site, and Building 14 renovations.

During the year, Mr. Joe Wilson was appointed to head the new Facilities Operations Unit which will operate and maintain the South Campus facilities. Several support positions were also filled. The hiring of qualified personnel is critical with the need to provide training, and establish procedures for operating and maintaining the new facilities. A major activity this year has been preparation of requisitions for spare parts, supplies and equipment.

Laboratory Services: Laboratory instrumentation services include design and fabrication of specialized instrumentation, fabrication of assorted laboratory paraphernalia, and repair and maintenance of laboratory equipment.

The unit, consisting of both the Machine and Electronics Shop, is completing in excess of 1200 work requests annually for the scientific staff plus many small repair work requests for RSB.

The Arts, Graphics and Photography Unit provides illustrations, graphics and photographic services to the Institute. During the past year, over 1000 work orders were processed. Because of the increased demand for services, the turn-around time for both graphics and photography has increased to 10 working days. In addition, a contract has been awarded to a local photographer for photographic processing services.

SAFETY OFFICE
Summary Statement

The Safety Office has the responsibility for all areas of safety and health at the Institute with the exception of radioistope usage.

The NIEHS Safety and Health Manual has been distributed to all program areas of the Institute. Thus far, a total of 205 copies have been distributed internally with an additional 60 copies going to outside requestors.

Safety training continues to be an important part of the overall safety program. This year over 250 individuals have participated in the various courses given by the staff of the Safety Office. Additionally, several investigators have taken a 2½ day course sponsored by NCI on the Safe Handling of Chemical Carcinogens in the Research Laboratory.

The proper disposal of hazardous chemical wastes has become a major problem that specific legislation had to be enacted to control it. The Resource Conservation and Recovery Act of 1976 which became effective this year strictly regulates the methods in which wastes can be transported, stored and disposed of and will have a great deal of impact on NIEHS. Pick-up methods and record-keeping have been changed to comply with the requirements of the act. Last year, 156 fifty-gallon drums of hazardous chemical waste was shipped from the Institute to an EPA approved burial site.

To aid in the record keeping requirements for all aspects of the safety program, the following computerized inventory and record systems have been developed with the assistance of the Biometry Branch:

1. Hazardous Waste Inventory - will file information on all wastes that are picked-up from the various areas of the Institute by compound, amount, investigator and method of disposal (EPA requirement).

2. Protocol Inventory - will file all hazardous agent protocols by agent/investigator(s)/location.

3. Noise Exposure Records - will provide for storage of noise dose data for employees in high noise areas (OSHA requirements).

4. Air Sampling Records - will store all workplace sampling data on selected workplace contaminants for individuals who have a potential exposure.

5. General Chemical Listing - will provide a listing of all chemicals in the Institute by laboratory/branch and building.

RADIATION SAFETY
Summary Statement

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, pickup 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.

There has been a dramatic increase in the amount of radioactivity used at the institute during the past year. In 1978, 1219 mCi of activity were received on site whreas in 1979, 3777 mCi were received. This increase has resulted in a heavy work load in many areas.

In addition to routine duties, the Radiation Safety Office has been involved with the Biometry Branch in developing a better computerized inventory system and has been actively writing some of its own programs for handling the bioassay data and dose calculations.

Disposal of low level radioactive wastes has become 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 actively investigating acceptable methods to incinerate more radioactive wastes and is working on monitoring procedures to insure that the environment is not degraded in any manner by increased incineration.

In July 1980, the Nuclear Regulatory Commission inspected NIEHS for compliance with its regulations. NIEHS was found to be in full compliance with the regulations except for two minor infractions which were immediately corrected.

LIBRARY
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 100 bibliographic data bases, maintenance of a collection of some 525 journal titles and 5000 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 2100 new books for the Library and the laboratories, publication of a monthly newsletter, and compilation of the annual bibliography of publications by Institute personnel. In FY 80, the Library published a new orientation brochure describing these functions.

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 100 data bases covering subjects from toxicology through business administation. During FY 80, Library personnel performed searches on 1000 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 remains 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 to try to cover as many of the diverse subject areas as possible which fall under "environmental health."

The Library subscribed to 92 new serials during FY 80, bringing the total to more than 525. In addition, the Library ordered about 250 subscriptions for the various laboratories. The Library continued its 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 NIEHS Library, working with EPA, produced a computer-generated journal holdings list updated for 1980.

Book Collection: Reflecting the decision to develop the book collection more thoroughly, 2100 books were purchased by the Library in FY 80. This was a 17% increase over the preceding year. About half of the books were purchased for the Library and about half for the Branches. Record keeping was simplified through a computerized on-order file.

Through the Federal Library Committee, the Library continued using an automated cataloging system, OCLC. OCLC is a computerized union catalog of books held by more than 1000 libraries nation-wide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for our new books which already have cataloging data on OCLC. At a push of the button the Library can order cards for the card catalog. As an additional benefit of

participation in OCLC, the Library gets a monthly print-out of the new books it has cataloged. This forms the core of the Library's newsletter and acquisitions list.

Another product from OCLC is a computer tape of the Library holdings. In another joint project with the EPA Library, the NIEHS Library developed a Computer-Output-Microfiche catalog. This catalog, composed of only ten microfiche cards, contains title, author, and subject catalogs for the entire NIEHS book collection. 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 tremendous increase in the number of photocopy and book requests in FY 80. The total of 14,250 was almost 50% more than in the previous year. Fortunately, 43% were filled by the Library from the in-house collection, an increase of 13 percentage points over the previous year, reflecting the improved NIEHS collection. Another 23% were filled by a student employed by NIEHS to do photocopying work at the Duke Medical Library. Only one-third of the requests had to be sent to other libraries, the main one being N.C State University.

The OCLC computerized catalog, mentioned above, also proved useful for verifying titles for interlibrary loan and for locating holding 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. A list of them was incorporated into the monthly newsletter. The Library published the 1979 NIEHS Bibliography, a catalog of the papers published by Institute personnel since 1966. With the aid of the Biometry Branch, author and keyword indexes were included for the first time. The Bibliography was distributed to NIEHS authors and to interested parties in other government agencies and in industry.

Planning: Planning for library and information services to meet the needs of an expanded organization and new facility continued in FY 80. A major accomplishment was the identification of a location for the Library in the new 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 set in motion. The addition of some temporary space in Building 18 was scheduled to take place in the fall of 1980.

The Librarian continued investigation of an integrated system for the automation of library functions. With the help of a feasibility study prepared by the Biometry Branch, development of an in house system was ruled out. In order to examine various options, the Librarian made trips to the first National On-Line Conference, the Army Pentagon Library and the NIH Library. He also represented NIEHS at a meeting of the Triangle Universities Libraries Cooperation Committee, a group directing plans for a computerized

catalog of the holdings of the Research Triangle university and research libraries. The Librarian tentatively concluded that NIEHS should join the NIH Library in its efforts to secure a total package. The functions to be included are: online catalog of book holdings, circulation records, acquisition files, and certain administrative operations. The NIH contract is not scheduled to go into effect until mid-1981; in the meantime, other options will be investigated.

Close contact with various library and information organizations was maintained by NIEHS in FY 80. The Librarian served on the nominating committees of the Special Libraries Association (SLA) Environmental Information Division and the North Carolina Chapter of SLA. He also served as Business Manager for the N.C. Chapter SLA Bulletin. The NIEHS Library hosted the winter meeting of the N.C. Online Users Group of information specialists.

INTRAMURAL RESEARCH

OFFICE OF THE SCIENTIFIC DIRECTOR

Annual project and budget review sessions with Laboratory/Branch Chiefs and their scientific staff have been instituted. This procedure provides an operational link between research achievement, planning, and the budget process. It also provides documentation for planning and an opportunity for researchers to participate in the process.

It has become clear that earlier plans to establish a Laboratory of Organ Function and Toxicology could not be realized with the limited funding available and the constraints placed upon appointment of research personnel. There was also reason to believe that considerable gains in research program coherence and scientific management could be achieved by consolidating three related small Laboratory units: Pharmacology, Pharmacokinetics, and the Organ Function and Toxicology Laboratory. Consequently, these Laboratories will be merged into the Laboratory of Pharmacology. This reorganization will be complete by the beginning of FY-81 and will result in a Laboratory with an outstanding record of scientific achievement and an improved structure for research planning.

The Intramural Research Council, which is composed of Laboratory and Branch Chiefs and the Scientific Director, has continued to perform extremely important scientific management functions. Revision of the By-laws of the Council have emphasized the role of this group in assuring the objectivity and equity in the selection of professional scientific staff in the Institute's Intramural Research Program. The procedures adopted by the Council for tenure and promotion actions largely anticipated those procedures which have now been institutionalized by the Deputy Director for Science and the Board of Scientific Directors, NIH. The participation of the Intramural Research Council in the management decisions of the Scientific Director's Office has been valuable and effective.

In January, 1980, the Board of Scientific Counselors reviewed the Laboratory of Animal Genetics and the Laboratory of Molecular Genetics. In June the Board reviewed three Laboratories: Environmental Biology, Organ Function and Toxicology and Reproductive and Developmental Toxicology. These reviews have been structured somewhat differently than in the past in order to provide small group interaction with the Laboratory scientists and to utilize increased participation of ad hoc expert peer reviewers in addition to the Members of the Board. This arrangement has proved to be successful. It relies on the Board for continuity of knowledge and experience with institutional matters and provides the scientific experts in the many scientific areas that cannot be covered by the restricted membership of the Board. These reviews are indispensable elements of scientific management and provide critical analysis of program achievement and structure. Two new members of the Board of Scientific Counselors, one in the area of neurobehavioral science and one in cell biology, are now in the process of appointment.

The projected retirement in July, 1981, of Dr. Alfred Edward as Chief of the Comparative Medicine Branch (Aniaml Facility and Care) has led to the formation of a search committee under the Chairmanship of Dr. Clifford Mitchell. A thorough search for a replacement has utilized representatives from the Intramural Research Program and the NIH Veterinary Resources Program. Two outstanding candidates have been selected for interviews and negotiations, which should lead to an appointment within the next few months, are now in progress.

The Inhalation Facility, which had previously been staffed and managed by the Environmental Biology Branch, has now been transferred to the Laboratory of Pulmonary Function and Toxicology. This facility, which is now in the process of upgrading exposure instrumentation, can be most effectively utilized in the context of the Pulmonary Toxicology Program. New research leadership in the area of lung pathophysiology is currently being recruited and several candidates have visited the Institute and explored the possibilities of appointment. This is a program of great importance to the mission of the NIEHS, and it is extremely encouraging to note the high quality of candidates whom Dr. Paul Nettesheim has interested in this position.

An Administrative Officer, Stillman Wright, has been appointed to the Office of the Scientific Director. The skillful conduct of the many matters of administrative management is necessary for the effective conduct of scientific research. Mr. Wright brings a wealth of experience and knowledge in these matters which has measurably improved the operation of the Office of the Scientific Director.

The Scientific Director continues to attend the bi-weekly meetings of the Board at NIH in Bethesda. With the appointment of Dr. Goldberger as the Deputy Director for Science at NIH, there has been considerable reexamination and change of policy and procedures. In order to maintain an informed position in this period, participation at these meetings has been important. Additional responsibilities in the administration of NIH have come through appointment to the NIH Performance Review Board for the administration of the new office of Personnel Management procedures, to the Board of the Fogarty Center and to the Selection Committee for NIH Lectures.

BIOMETRY BRANCH

The Biometry Branch fulfills a dual role within the Institute's Intramural Research Program. Its primary emphasis is on the initiation of applied, environmental-health oriented research in the areas of biomathematics, risk assessment, and epidemiology. In addition the Branch has responsibility for providing statistical, mathematical, data processing, systems development and computer engineering support to the Institute.

Biomathematics research is concerned with the mathematical modelling for biological systems and involves collaboration with a variety of other research groups at the Institute. Within this research effort attention continues to be centered on the areas of mathematical population genetics, pharmacokinetic modelling, and methodology development for bacterial assays. Risk assessment activities have emphasized improved utilization of data generated from high-dose-level animal screening studies to estimate long-term human risk associated with low environmental levels of exposure. Research is being conducted on a variety of issues that relate to different aspects of this general problem area. The Branch's epidemiology program is concerned with the identification of potential environmental components of chronic disease etiology. In addition a major effort is expended on assessing the impact on reproduction and human health of exposure to hazardous agents in the environment. Research activities in these areas range from analyses based on secondary data bases to the initiation of direct field investigations. Methodological research related to the development of laboratory methods and statistical techniques in support of epidemiological studies is also conducted.

Statistical support provided to the intramural program covers a wide range of consulting activities from experimental design and data analysis to model estimation and simulation studies. An active research effort in the development of new analytic techniques is maintained in order to meet some of the highly specialized needs of various Institute scientists. Computer support covers such diverse activities as the creation of information retrieval and inventory maintenance systems, the simulation of complex biological models designed to gain insight into mechanistic processes, and the processing of large volumes of data in conjunction with statistical consulting activities. In addition, computer engineering support is provided for real-time data acquisition and minicomputer controls systems.

BIOMATHEMATICS

Work is currently underway in three separate areas of mathematical biology. All of these programs involve collaboration with other research groups within the Intramural Research Program. Joint research with the Laboratory of Animal Genetics is directed toward developing mathematical models for phenomena in population genetics. Improvements have been made on an earlier model for estimating nucleotide mutation rates from restrictive enzyme map data. Simple computational formulas have been developed, and the model has

been modified to handle complete DNA sequence data. New research has been initiated to develop models for the evolution of transposable DNA elements.

Joint research with the Laboratory of Pharmacokinetics is directed towards the development of pharmacokinetic models and methods for quantifying the mechanisms of xenobiotic uptake, distribution, metabolism, and excretion in animals and man. Physiological compartmental models of the in vivo chemokinetics of several halogenated biphenyls have been constructed for the rat and efforts to scale these models to predict the fate of these compounds in other animal species are in progress. Collaboration with the Marine Pharmacology and Biomedicine Program in the Laboratory of Pharmacology has resulted in a preliminary model for the pharmacokinetics of selected organic pollutants in a marine animal, the spiny lobster. This latter work is aimed at better understanding the potential accumulation of toxicants in human marine food sources.

Efforts are underway to adapt and improve existing models that are used to predict the number of mutants in growing bacterial populations. These models have become increasingly more important because of the current interest in the Ames test and other in vitro test systems that are used for detecting chemical mutagenesis. Currrent work is aimed at incorporating toxicity into the models and relating it to dose response curves.

RISK ASSESSMENT

The major focus of risk assessment research is on the development and/or modification of statistical procedures for estimating long-term human risk resulting from low-level exposure(s) to chemical carcinogens using data generated from animal carcinogenicity tests conducted at high, often maximum tolerated dose levels. A variety of research projects relating to different aspects of this problem are currently underway within the Branch.

The degree to which low dose extrapolation is altered by replacing the administered dose with the amount of DNA-carcinogen adduct formed was evaluated and found to be highly model dependent. Efforts are now being made to identify those pharmacokinetic parameters which exert the most influence on the low dose risk estimates.

Research was conducted on a modified (and more realistic) version of the traditional competing risks problem. In this study it was assumed that cause-of-death could not be stated with certainty, even though some probability of occurrence could be assigned to each of the causes under consideration. A maximum likelihood estimation of survival time was developed for this model.

Investigation of the various factors influencing species-to-species extrapolation were continued, with some consideration being given to non-oncogenic endpoints.

Attempts have also been initiated to modify the design of the current lifetime cancer bioassay so as to improve its low dose extrapolation potential

without seriously sacrificing its ability to identify possible carcinogenic chemicals.

EPIDEMIOLOGY

Field studies of human disease, particularly chronic disease, due to environmental pollutants continue to play a major role in the Biometry Branch's epidemiology program. Attention is also directed toward basic and applied research in laboratory support methodology, and to some extent in biostatistics.

A prospective study, the Breast Milk and Formula Project, is now in its third year. This study follows the growth, development, and morbidity of about 900 children from birth, and includes measures of organohalide contaminants (polychlorinated and brominated biphenyls, DDT and its metabolites) in the mothers' serum and breast milk. During this year, plans for five year follow-up were completed.

Preparations for a case-control study of spontaneous abortion are now coming to a close with the initiation of a contract for the development of a research survey questionnaire for mothers and fathers involved in an early spontaneously aborted pregnancy. This questionnaire can also be utilized in other studies of pregnancy and negative reproductive outcomes. One recently completed study in this area examined the secular trend for spontaneous abortion over a period of more than forty years in two cohorts of university women who self-reported their menses and pregnancy outcomes. In a related investigation using a similar data set, the effects of certain confounding factors including maternal age and gravidity will be studied with respect to spontaneous abortions. A mathematical model for pregnancy outcome is being developed and will be compared with empirical data. A study of spontaneous abortion among perchlorethylene-exposed dry cleaning workers in a mid-western city will attempt to test the hypothesis that this suspect carcinogen/mutagen is in fact responsible for increasing the rate of negative reproductive outcomes when compared with a similar group of unexposed individuals. A protocol for that study has been completed and is undergoing peer review. The study itself is scheduled to begin in 1980.

A major investigation of reproductive effects involves workers and their conjugal partners exposed to low levels of ionizing radiation at a nuclear power facility. A series of four epidemiologic studies are intended to evaluate the results of such radiation on human reproductive function. The endpoints of interest include birth defects, spontaneous abortion, fetal and infant death, childhood cancer, and growth and development. The populations under study include nuclear workers who are occupationally exposed, and a cohort of births originating from these workers.

In the laboratory area, several general projects have begun. One effort is concerned with the detection of human exposure to mutagens by analyzing body fluids including urine, plasma, and tissue specimens. A second project involves the development of laboratory methods and procedures for assessing genetic damage to human tissues after exposure to toxic substances. Furthermore, an epidemiologic study of the role of Schistosomiasis haematobium

in the pathogenesis of bladder cancer in Egypt will be undertaken; and laboratory components of that study will attempt to assess the presence and role of urine mutagens in the development of bladder cancer.

Another area of program activity is metal and trace element toxicity. A study on enzyme markers for susceptibility to the hematologic effects of lead has continued, and a study of the role of selenium deficiency and other trace elements in the production of skin cancer is planned.

A number of investigations of various chronic disease endpoints will also be initiated. Potential underlying etiologic factors of chronic renal disease will be evaluated in a clinic-based case-control study performed collaboratively with a university medical center. In addition two other cancer epidemiology studies are also planned. One will try to assess the role of parental smoking in the development of cancer in offspring; and the other will attempt to test the hypothesis that occupational exposure to hair dyes is a risk factor for the development of breast cancer in female beauticians. Finally, an interagency agreement with FDA and CDC for the study of the association between aflatoxin B and Reye's Syndrome is in place.

STATISTICAL CONSULTING

The Biometry Branch provides a comprehensive statistical consulting service for the Intramural Research Program. This effort covers a wide range of activities in the areas of experimental design and data analysis.

In the area of experimental design one of the most frequent problems involves sample size determination. This is an important issue since it is essential before an experiment is undertaken to assess the feasibility of achieving the study objectives with the available resources. Consideration is also given to possible improvements in the basic design itself, so as to eliminate possible confounding factors and to insure optimum allocation of animals.

The Biometry Branch also provides data analysis support, including tabulation of summary statistics, curve fitting, significance testing, and interpretation of test results. These efforts are closely co-ordinated with the computing work group.

Frequently, applied statistical research results as a direct consequence of the Branch's consulting activities. For example, a need for improved statistical methodology in the area of short-term mutagenicity testing led to (1) the development of statistical methods for detecting departure from the Poisson model, and (2) a comprehensive study of the relative power of several k-sample trend tests. In the area of toxicological data analysis the feasibility of using randomization tests for binary response data is being studied. Finally, the Breslow and Cox procedures are being compared under several assumed underlying models for the analysis of tumor data from long-term cancer bioassays.

COMPUTING

The Biometry Branch has the responsibility of providing computing and data processing support to NIEHS. The activities accomplishing this support may be thought of as consisting of two cooperating and interlocking efforts, namely computer operations and programming, 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.

Of major importance to the data processing effort this FY has been the continued provision of ten contract analysts, programmers, and data entry personnel obtained through GSA/IDSF. This has allowed the development of software and systems which would not have been otherwise possible in view of our shortage of slotted positions. Two other activities which will ultimately lead to the existence of major systems involve the provision of consulting and systems analysis support to (1) the Comparative Medicine Branch for its microbiology and animal facility systems and (2) the Environmental Mutagen Test Development Program for its test compound information system. Additionally, information systems are being developed to serve the NIEHS administrative offices and to provide information management services to the National Toxicology Program. In direct support of the Biometry Branch's statistical data analysis activities, Penn State's "MINITAB" interactive statistical analysis package has been installed on the PDP 11/70. It is expected that improvements and extensions to this system will be undertaken during the next FY.

Development of the capability for providing computer engineering support to the laboratories of the Institute is also ongoing within the Biometry 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. The following efforts are underway or were completed in the last year: (1) design support of computer interfaces; (2) computer system support of the development of a scanning microscope for biochemical genetics studies; (3) development of a multipurpose lab minicomputer adaptable to population genetics studies; (4) development of minicomputer systems for the acquisition of scintillation counter data in support of diverse studies; (5) computer hardware support of the growth of the Biometry Branch central computer (PDP 11/70); (6) assisted in the design of a balance interface that links an electronic balance and standard terminal to a computer using a single serial line; and (7) continued development of a number of on-line laboratory inventory software systems. In addition, a variety of computer support has been provided for the Laboratory

of Behavioral and Neurological Toxicology. Most of this support has been software development including a variety of (1) data acquisition and experiment control, (2) manual data entry, (3) data reduction and reporting, (4) data base, (5) local data analysis, and (6) data communications software. General computer consulting support was also provided for a joint NIEHS/NCHS data communications link to the Washington area, a future animal inventory and weighing system, and various terminal acquisitions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40002-10 BB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Barry H. Margolin	Mathematical Statistician	BB	NIEHS
	Beth C. Gladen	Staff Fellow	BB	NIEHS
	Joo O. Koo	Visiting Fellow	BB	NIEHS
	Amy H. Poon	Visiting Associate	BB	NIEHS
	Clovis A. Peres	Visiting Associate	BB	NIEHS
	Robert I. Jennrich	IPA	BB	NIEHS
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COOPERATING UNITS (if any)

LAB/BRANCH

Biometry 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 response data, survival analyses, detection of aberrant observations, nonlinear least squares procedures, and dose-response methodology.

PHS-6040
(Rev. 10-76)

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) Methods to detect departure from the Poisson model have been investigated, with particular emphasis on a negative binomial alternative; these statistical procedures have immediate applicability to the modeling of short-term mutagenicity assays. (2) Procedures for the detection of outliers, i.e. aberrant observations, in an otherwise multivariate normal data set have been studied and a procedure with certain optimality properties has been obtained. (3) Some local convergence therorems for the generalized secant Gauss-Newton algorithm (a popular derivative-free method of nonlinear least squares estimation used in fitting pharmacokinetic models) were derived. These thoeorems assert convergence for the algorithm on small residual problems and superlinear convergence on zero residual problems. (4) The use of generalized least squares in estimating mean and variance components in the general mixed model is being studied. This investigation also addresses the problems involved with handling non-negativity constraints for variance components. (5) The nonlocal asymptotic optimality of the likelihood ratio statistic is being studied for suitable composite hypotheses when the test is performed conditionally given a sufficient statistic. Asymptotic optimality is being considered in the framework of Bahadur efficiency. (6) The use of randomization procedures for the analysis of binary response data is being investigated and the power of these procedures is being compared to that of alternative nonparametric techniques. (7) A Monte Carlo study of the power of some k-sample tests for ordered binomial alternatives was carried out. It was found that for small group sizes exact tests based on isotonic regression and Armitage's test for linear trend had similar power. However, exact tests appear to be less sensitive to reverse alternatives and more powerful for larger group sizes than the Armitage procedure. (8) Comparisons were made of Pearson product moment correlation, Winsorized correlation and Blomquist's quadrant measure under assumptions of bivariate normality, and contaminated bivariate normality. Pearson's correlation coefficient compared unfavorably with the other two measures for heavily contaminated distributions. (9) Monte Carlo studies are continuing to compare the relative power of the Cox and Breslow survival analyses procedures under a variety of conditions.

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

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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, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40004-03 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	BB	NIEHS
Other:	Walter J. Rogan	Medical Officer	BB	NIEHS
	Michael D. Hogan	Mathematical Statistician	BB	NIEHS
	Sylvaine Cordier	Visiting Associate	BB	NIEHS
	Hirofumi Takagi	Visiting Fellow	BB	NIEHS
	Allen J. Wilcox	Medical Officer (Research)	BB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's activities in the field of epidemiology. The objectives are both to broaden understanding of the uses and limitations of currently employed study designs and corresponding analyses; and to develop new techniques for statistical analyses of epidemiological studies.

-0040
(Rev. 10-76)

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) A computer program to assess feasibility of cohort studies was developed. The program computes the minimum detectable increase in risk for a proposed study design. The input to the program consists of quantities the investigator is actually likely to know, such as the available sample size and the sex composition of the study groups. Background mortality and disease rates are computed from standard rates. (2) An investigation of the use of path analysis in epidemiology was undertaken. The possiblity of using path analysis to investigate causal relationships was suggested. (3) Preliminary work suggests that the design of past studies of spontaneous abortions may have caused bias which clouds the interpretation. Work was begun on models to account for this bias and estimate its magnitude. (4) Investigation of the possible effects of matching on case-control studies continued.

The proposed course is to continue research efforts on statistical methodology problems that arise in connection with the epidemiological investigations carried out by the Biometry Branch.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 40005-03 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Norman Kaplan	Research Mathematician	BB	NIEHS
	Bruce Collings	Mathematical Statistician	BB	NIEHS
	Errol Zeiger	Head, EMTDP	LMG	NIEHS

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics

LAB/BRANCH
Biometry Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The long-term objective of this ongoing project is to develop appropriate statistical techniques for the analysis of data arising from the Mutagenesis Testing Program. Focus has been on data from microbial test systems. Statistical procedures currently employed by other researchers in mutagenesis for the design and analysis of microbial experiments have been studied, and new and improved procedures continue to be devised.

FORM-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Test for mutagenicity remains under study from a statistical standpoint. Stochastic modeling of this test has progressed to permit the inclusion of toxicity as a competing risk vis a vis mutation for each microbe plated.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The new family of models for Ames Test data yields analyses of mutagenicity that are far more resistant to the presence of toxicity than those previously proposed. (2) Differences among laboratories in the variability of their Ames Test data has been documented; this feature has been incorporated into the model of the Ames Test, and will enable application of quality control procedures to the ongoing operation of a laboratory performing this assay. (3) A file of over 100 experiments performed on contract to NIEHS is in the process of being analyzed via the Ames Test model to develop a sense of whether further refinements are needed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project has altered the ways in which microbial 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 microbial tests. This research effort is yielding methodological results of interest to biometricians in numerous other areas of research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41001-06 BB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Michael D. Hogan	Mathematical Statistician	BB	NIEHS
	Norman L. Kaplan	Research Mathematician	BB	NIEHS
	Clovis A. Peres	Visiting Associate	BB	NIEHS
	Amy H. Poon	Visiting Associate	BB	NIEHS
	Christopher Portier	Mathematical Statistician	BB	NIEHS
	Marshall W. Anderson	Mathematician	LPK	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacokinetics

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	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 main objective of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long-term human risk from chemical carcinogens at very low dose levels. Current research efforts are focusing on a variety of issues such as competing risk analysis, species-to-species extrapolation, the incorporation of pharmacokinetics in low dose risk estimation, and cancer bioassay design. In addition mathematical models for the genetic effects of radiation have also been considered.

FS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) A general scheme has been studied which relates carcinogenic response to the amount of DNA-carcinogen adduct formed instead of the applied dose. (2) The evaluation of factors that can influence/explain species differences in response to potential carcinogenic agents is being continued, with consideration also being given to non-carcinogenic endpoints. (3) Research is being conducted on a modification of the traditional competing risks problem in which only the time to death and the probability that death can be attributed to the cause-of-interest is known. (4) Attempts are being made to modify the standard cancer bioassay in order to improve its potential for low-dose extrapolation and risk estimation. (5) The two-component and quadratic dose-response models for genetic effects of radiation and the biological assumptions upon which they are based were evaluated.

MAJOR FINDINGS AND PROPOSED COURSE (1) Even though the incorporation of pharmacokinetics into the low dose estimation process better reflects reality, the results of the current research effort show that the estimates are still very sensitive to which underlying model is used for the extrapolation. Work is also underway to determine which pharmacokinetic parameters have the largest effect on the low dose estimates. (2) A maximum likelihood estimator for the survival function in the modified competing risk situation (described above) was derived. The estimator has been shown to be asymptotically normal; and Monte Carlo simulations seem to indicate that in small sample situations it compares favorably (w.r.t. bias) to its traditional competitor for tumors ranging from early to late onset. (3) Research into the cancer bioassay will continue, emphasizing the development of a design that will minimize the variance of the estimated excess risk and still yield sufficient power for carcinogenicity testing or screening. (4) The comparison of the extrapolation models for radiation-induced genetic change suggested that the quadratic model is preferable for a lethal endpoint, while the two-component model should be employed for a response like point mutations.

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 procedures for realistically assessing this risk is obvious.

PUBLICATIONS

Hoel, D.G.: Animal experimentation and its relevance to man. Proceedings from US-Japan Conference on Biostatistics in the Study of Human Cancer, May 1978. Environmental Health Perspectives 32: 25-30, 1979.

Hoel, D.G.: Sequential methods in genetic risk assessment. Genetics 92: 196-198, 1979.

Hoel, D.G.: Statistical approaches to toxicological data. Proceedings, 5th Symposium on Statistics and the Environment, NAS. Environmental Health Perspectives 32: 267-271, 1979.

Schoenfelder, C.A., and Hoel, D.G.: Properties of the Neyman-Scott carcinogenesis model at low dose rates. Mathematical Biosciences 45: 227-246, 1979.

Hoel, D.G.: Incorporation of background in dose-response models. Proceedings from Symposium on Extrapolation of Laboratory Toxicity to Man: Factors Influencing the Dose-toxic Response Relationship. Federation Proceedings 39(1): 73-75, 1980.

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. Journal of Toxicology and Applied Pharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 42002-08 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacokinetic Modeling and Methodology Development

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

PI:	Daniel B. Tuey	Staff Fellow	BB	NIEHS
	Hazel B. Matthews	Research Chemist	LPK	NIEHS
	Marshall Anderson	Mathematician	LPK	NIEHS
Other:	Robert L. Dedrick	Section Head	BEIB	DRS
	Robert J. Lutz	Chemical Engineer	BEIB	DRS

COOPERATING UNITS (if any)
Chemical Engineering Section, BEIB, DRS, NIH
Laboratory of Pharmacokinetics

LAB/BRANCH
Biometry Branch

SECTION

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 purpose of this project is to develop pharmacokinetic models and methods for quantitating the mechanisms of the uptake, distribution, metabolism, and excretion kinetics of environmental xenobiotics in various animal species. Mathematical models of the in vivo pharmacokinetics of several halogenated biphenyls in the rat have been developed and have been scaled to the mouse. Other models have been constructed to describe the disposition of these environmental contaminants in animals following chronic exposure. The pharmacokinetics of xenobiotics in marine animals that serve as human food sources is also being modeled. These mathematical models are intended to provide functional strategies for helping to extrapolate the disposition of chemical contaminants in the environment from one compound to another, and from one animal species to another, ultimately to include man. The evaluation and implementation of existing techniques and the development of new methodologies needed to accomplish these objectives are an integral part of this project.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The physiologic modeling approach used in drug disposition is being employed to develop similar type models for environmental contaminants. Computational algorithms for solving the equations that describe these models are being implemented.

MAJOR FINDINGS AND PROPOSED COURSE: (1) As for the rat and the mouse, physiological compartmental models have been constructed to describe the individual disposition kinetics of several different polychlorinated biphenyl (PCB) compounds in the dog and monkey. The tissue-level time-courses predicted by the models were generally consistent with the experimental data. Skin and other tissues may not be perfusion-limited as assumed in the present model. Metabolism is the rate-determining step for the elimination of these compounds. The dog is able to metabolize and excrete 2,4,5,2',4',5'-hexachlorobiphenyl rapidly compared to other mammals studied to date. Attempts to extrapolate PCB pharmacokinetics between species, and from chlorinated biphenyl to another, are in progress. (2) When allometric equations were used to scale the model for the rat to predict disposition kinetics in the mouse, good results were obtained for the more persistent, slowly metabolized congener. Whole body elimination kinetics after a single i.v. dose to mice was used to predict the body burden in other mice that received multiple oral doses of the same PCB compound. (3) A physiological compartmental model constructed from data obtained from rats that received a single i.v. dose of 2,4,5,2',4',5'-hexabromobiphenyl was able to simulate and predict the tissue levels found in rats placed on a 30-day chronic oral dose schedule. The model has been scaled to man and used to estimate nominal past and probable future body burdens associated with current human blood and adipose tissue levels of PBB. (4) A blood flow-limited physiological pharmacokinetic model was used to describe the overall time-course of octane and hexadecane disposition kinetics in the lobster, which possesses an open circulatory system. This research revealed that the crustacean shell is an important storage depot for these compounds. In related work, compartmental analysis was used to infer that 2,4-dichlorophenoxyacetic acid (2,4-D) and related herbicides are excreted via an organic anion active transport system in the lobster green gland, and a quantitative estimate of the relative importance of metabolic conjugation was obtained. (5) A commercially available computer software package for simulation and identification of pharmacokinetic models was evaluated.

Future work will include attempts to refine the PCB models and account for small discrepancies which may prove significant in long-term studies. Functional relationships for extrapolating the pharmacokinetics of PCBs among the rat, mouse, dog, and monkey using the physiological compartmental models and in vitro estimates of metabolism rates will be tested. Compound-to-compound extrapolation of long-term PCB disposition kinetics based on short-term excretion kinetics and chlorination pattern will also be investigated. Mathematical models will be developed for the pharmacokinetics of additional aquatic contaminants and in other marine animals. The impact of pharmacoki-

netic considerations on the use of experimental test systems to assess human exposures and risks at environmental levels will be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An ability to quantitate pharmacokinetic similarities and differences from species to species and from compound to compound will enable better judgements to be made of the relevance of animal study results to man.

PUBLICATIONS

Morales, N.M., Tuey, D.B., Colburn, W.A., and Matthews, H.B.: Pharmacokinetics of multiple oral doses of selected polychlorinated biphenyls in mice. Toxicology and Applied Pharmacology 48: 397-407, 1979.

Matthews, H.B., and Tuey, D.B.: The effect of chlorine position on the distribution and excretion of four hexachlorobiphenyl isomers. Toxicology and Applied Pharmacology 53: 377-388, 1980.

Tuey, D.B : Toxicokinetics. In Hodgson, E. and Guthrie, F.E. (Eds.): Introduction to Biochemical Toxicology. New York, Elsevier, 1980, pp. 40-66.

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. Toxicology and Applied Pharmacology 53: 420-431, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43001-08 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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)	BB	NIEHS
	Michael D. Hogan	Mathematical Statistician	BB	NIEHS
Other:	Anna Bagniewski	Mathematical Statistician	BB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.00

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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.

-1040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Age, race and sex-specific liver cancer death rates were calculated for liver cancer deaths between 1958 and 1975 in the United States. Time trends, cohort trends, and sex and race differences in liver cancer mortality were examined.

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 only. There were no corresponding increases over time for any other sex/race group, and in fact, rates for white females tended to decline from 1958 to 1975.

Future analyses will be conducted to determine if these relationships can be explained by differences in exposure to suspected liver cancer risk factors. In particular, analyses will be conducted using reported cases of hepatitis B and published data on alcoholism and/or alcohol consumption to see if these factors are associated with liver cancer mortality. Correlational analyses will be conducted for liver cancer deaths in the United States and for 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 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43002-04 BB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Beth C. Gladen	Staff Fellow	BB	NIEHS
	James D. McKinney	Supervisory Chemist	LEC	NIEHS
	Douglas B. Walters	Chemist	LEC	NIEHS
	Anna Bagniewski	Mathematical Statistician	BB	NIEHS
	Richard Everson	Epidemiologist	BB	NIEHS

COOPERATING UNITS (if any)

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

Biometry 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 to PCB's.

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 placental 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 are performed over the first years of the child's life including behavioral evaluations, at birth and one at 6, 12, 18, 24 months, and then yearly.

MAJOR FINDINGS AND PROPOSED COURSE: No data analysis is yet complete. Data collection in North Carolina will continue. Major progress to date has been enrollment of about 2/3 of 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; and (5) to establish a cohort of children for follow-up studies. (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.

PUBLICATIONS

Rogan, W.J., Bagniewski, A.B., and Damstra, T.: Pollutants in breast milk. New England Journal of Medicine, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43003-02 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Beth C. Gladen	Staff Fellow	BB	NIEHS

COOPERATING UNITS (if any)

Medical University of South Carolina

LAB/BRANCH
Biometry 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. We have begun investigation into genetic differences in susceptibility to the effects of lead on blood formation.

PHS-6040

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 are now constructing a stratified sample for revisit. They will find the children and do the blood work. Data gathering should be complete early in 1981, and analysis late in 1981.

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 have treated children with undue lead absorption. The decision to treat is made on the basis of laboratory tests rather than clinical illness, and thus it is likely that some children are treated unnecessarily. Successful identification of children with greater susceptibility would allow more effective screening for children likely to show toxicity.

PUBLICATIONS

O'Tauma, L.A., Rogers, J.F., and Rogan, W.J.: Increased lead absorption in children of battery workers. Journal of the American Medical Association 24(18): 1893, 1979.

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, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43004-02 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	Stephen M. Brown	Medical Officer (Research)	BB	NIEHS
Other:	Richard B. Everson	Epidemiologist	BB	NIEHS
	Dale P. Sandler	Statistician (Health)	BB	NIEHS
	Walter Rogan	Medical Officer	BB	NIEHS
	Allen J. Wilcox	Medical Officer (Research)	BB	NIEHS

COOPERATING UNITS (if any) School of Public Health, University of North Carolina, Chapel Hill, N.C., School of Public Health, University of California, Berkeley, Cal., Wilson Dermatology Clinic, Wilson, N.C., Food and Drug Administration, U.S.F.D.A., Center for Disease Control, U.S.C.D.C.

LAB/BRANCH
Biometry 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 related 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 childhood cancer; an investigation into the relationship of selenium and other trace elements to skin cancer; an investigation of occupational exposure to hair dyes and the occurrence of death from breast cancer, and an investigation of the relationship of estrogen receptors to other known risk factors for breast cancer.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: The central objectives of this study are to develop refined techniques for chronic disease environmental epidemiology and to utilize these methods in studies of influences on frequency of cancer and other chronic disease. The collection of data for the Epidemiology Program will be from a variety of sources in the several components of this broad project area. (1) Tissue samples from children dying of Reye's Syndrome, of other liver disease, or of other causes (10 each) will be studied. Specimens obtained by CDC will be analyzed by FDA for aflatoxin B. Mass liquid chromotography and mass spectrophotometry will be utilized, with pathologic confirmation of disease and analysis of data at NIEHS. (2) A case control study of more than 300 skin cancer patients in Wilson, North Carolina will utilize a survey instrument and neutron activation study of blood and serum samples to determine the association if any between six trace elements and the occurrence of forms of this disease. (3) Using data already collected, an investigation will test for association between known risk factors for breast cancer such as age at first birth, parity, age, etc. and levels of estrogen receptors in tumor tissue. (4) A case control design will be used to examine maternal smoking during pregnancy as a risk factor for the occurrence of cancer in the offspring of such persons, when those offspring reach adulthood. Persons with cancer (cases) and friends without cancer (controls) will be interviewed to determine their exposure to cigarette smoke from a variety of sources including maternal and paternal smoking prior to and/or during pregnancy. 550 cases and matched controls of equal number will be studied. (5) Using available mortality data and death files for control, all cases of breast cancer in a population base of a million persons for a peri-censal period will be compared with a set of two matched controls for each case, to determine if the occupation beautician is associated with increased risk of death from this disease. Existing mortality dated tapes for cancer in Alameda County, California and accessibility of control data permit the assessment of this association with relative ease.

MAJOR FINDINGS AND PROPOSED COURSE: (1) After a series of conferences, collaborative arrangements have been made with two other agencies (CDC, FDA) to executive this protocol in FY 81. (2) An interview instrument has been developed and collaborative arrangements made with clinical facilities for performance of interviews, physical examinations, and the collection of blood samples; the actual survey process has been begun. In addition, collaborative arrangements have been made with a laboratory which is to perform trace elements analysis. This project corresponds with Project #Z01 ES 43004-01 BB in the 1979 NIEHS Annual Report. (3) Data has been obtained on the estrogen receptor level and a number of other risk factors relating to breast cancer, in order to achieve this analysis. (4) A protocol has been written for the testing of this hypothesis, this study being in a preparatory phase, scheduled to commence approximately July 1, 1980. (5) A data tape has been obtained making available all breast cancer cases in the population noted; a written protocol has been written and reviewed. A procurement agreement has been

executed with a collaborating institution where the listing of cases and designation of control as well as other clerical and computer analytic tests will be performed and work to be completed before the end of FY 80.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE:
Etiologic factors related to environmental exposure of persons to carcinogens or mutagens and risk factors associated with them is of great significance to the objectives of epidemiologic research within NIEHS. These efforts are in part coordinated with the establishment of intramural laboratory methods and other program efforts related to congenital, genetic, and/or prenatal conditions which are also likely to be sensitive to or etiologically related to mutagenic agents.

(1) Several reports pointed to association between a viral agent (Varicella or Influenza A or B) with a drug or chemical in the etiology of Reye's Syndrome. A systematic testing of the hypothesis that aflatoxin B is such a chemical will permit great progress in understanding the significance of this often lethal chronic disease of children.

(2) The role of selenium, cadmium, and other trace elements in either protecting against or predisposing toward the occurrence of cancer will be examined with respect to measured exposure and historical occupational exposure to such elements, in the context of a case control design. Interaction between chemical substances and other known environmental causes of skin cancer (e.g., ultraviolet rays) will have an important effect both in illucidating the potential role of trace elements in human cancer, and the possible phenomenon of cynergism in producing such occurrences.

(3) The phenomenon of estrogen receptors is one particular area in which an etiology for breast cancer has become known or suspect in relationship to external or endogenous stimuli of a chemical nature. Thus the detection of an association between estrogen receptors (which are also known to be receptive to certain environmental chemicals) and other breast cancer related risk factor would shed additional light on the etiology of this important disease.

(4) Studies of parental smoking and cancer in children are homologous or in fact paradigms for studies of transplacental carcinogenesis in man. This study would establish the possibility that environmental maternal or paternal exposure may be carcinogenic for offspring. Since such carcinogenesis has been demonstrated repeatedly for mammalian species other than man, it is most important to seek further human evidence. Approximately 15% of persons under age sixty with cancer would have mothers who were smokers; more than 40% of children born today are born with smoking mothers. This investigation is therefore of potential public health significance as well as having bearing on basic understanding of transplacental or genetically mediated, carcinogenesis.

(5) Animal studies and mutagenesis test data point towards a relationship between a number of oxidative permanent hair dye chemicals and the occurrence of cancer or mutation. There is a significant set of human data relating occupational exposure by beauticians to hair dye as with the occurrence of bladder cancer and lung cancer. While hair dye users have been shown to have certain risk of breast cancer, no occupational studies have been published to establish such an association. This small study using existing data would therefore greatly amplify the meaningfulness of the latter studies, and public health significance of exposure to such compounds.

PUBLICATIONS

Barr, M., Jr., Keller, C. A., Rogan, W. J., and Kline, J.: Summary of the workshop on perinatal and postnatal defects and neurological abnormalities from chemical exposures. Annals of the New York Academy of Sciences 320:458-472, 1979.

Brown, S. M.: The use of epidemiologic data in the assessment of cancer risk. Journal of Environmental Pathology and Toxicology, in press.

Rogan, W. J.: Advances in prevention environmental and occupational medicine. In Arnold, C. (Ed.): Annual Progress in Preventive Medicine. 1979, in press.

Rogan, W. J.: Analytical chemistry needs for environmental epidemiology. In McKinney, J. (Ed.): The Chemistry of Environmental Agents as Potential Human Health Hazards. Ann Arbor, University of Michigan Publishers, in press.

Rogan, W. J.: The sources and route of childhood chemical exposures. Journal of Pediatrics, in press.

Rogan, W. J., and Rall, D. P.: The National Toxicology Program and the pediatrician. Journal of Pediatrics, in press.

Everson, R. B.: Identification of population sensitive to the impact of the byproduct of technology transfer. Proceedings of the Symposium of the Biomedical Impact of Technology Transfer, Cairo, Egypt, February 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43006-02 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of Reproductive Effects of Low-Level Radiation Exposure

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)	BB	NIEHS
Other:	Stephen M. Brown	Medical Officer (Research)	BB	NIEHS
	David G. Hoel	Chief	BB	NIEHS

COOPERATING UNITS (if any)
Battelle Pacific Northwest Laboratories, Richland, Washington
Hanford Environmental Health Foundation, Richland, Washington

LAB/BRANCH
Biometry Branch

SECTION

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)

A series of four epidemiologic studies are intended to evaluate the possible effects on human reproduction of low-dose radiation exposure. The individual studies are designed to specifically examine the relationship of radiation exposure to birth defects, spontaneous abortion, fetal and infant death, childhood cancer and human growth and development. Each of the studies focuses on different possible reproductive outcomes, but the studies and the study populations overlap and will provide complementary data. Two general populations are being studied. These are a cohort of nuclear workers (female workers and wives of male workers) who are occupationally exposed to low levels of radiation (external and internal exposures), and a cohort of births to nuclear workers. Data for all segments of the study will be collected using medical records, vital records, subject interviews, employment records, or a combination of these sources.

(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these studies is to examine reproductive outcome in workers exposed to low-level radiation. The hypothesis is that risk of adverse pregnancy outcome will vary with different levels of low-dose radiation exposure.

METHODS EMPLOYED: Two study cohorts have been identified. These are a cohort of Hanford workers (and their pregnancies) and the cohort of births to Hanford workers in a 20-year period. A well-defined historical cohort of approximately 3,500 workers will be traced to the present time. Detailed reproductive histories will be obtained from this cohort (or from spouses, for male workers) during a telephone or home interview. Reproductive outcomes will be compared for workers with varying levels of radiation exposure. The birth cohort will be studied in several ways. A case-control study of birth defects and fetal and infant deaths will be conducted, using cases identified from the cohort of Hanford births. Approximately 400 cases and 400 controls will be studied. Radiation exposures will be compared for parents of cases and parents of controls (normal infants or children). Factors of the newborn period (such as birthweight and head circumference) will be studied for approximately 3,000 Hanford births during a ten-year period. Again, "outcome" measures will be compared for exposed and non-exposed births and also by level of radiation exposure. The health and development of a sample of approximately 1,000 exposed and non-exposed babies will be followed, retrospectively, through age eight. Data for all segments of the study will be collected using medical records, vital records, subject interviews, employment records, or a combination of these sources.

MAJOR FINDINGS AND PROPOSED COURSE: Detailed study proposals have been reviewed by non-institute scientists. The necessary forms clearances and Human Subjects Committee approvals are currently being obtained. The entire study is expected to take 42 months to complete. Individual studies will be phased in, beginning with the study of spontaneous abortion among members of the worker cohort and the case-control study of birth defects, which are both beginning in the fourth quarter of this year. Results from the case-control study will be available no earlier than July, 1981. Results from the other studies will follow at a later date. The reproductive studies are being conducted through an Interagency Agreement with the Department of Energy, and through them, Battelle Pacific Northwest Laboratories and the Hanford Environmental Health Foundation. Agreement Number 222 Y01-ES-00049 is for a period of 42 months, at an estimated total cost of $935,000. Costs for FY 80 will not exceed $200,000. A total of 1.75 person-years were supplied by the Agreement in FY 80 (0.25 professional and 1.0 Other). Actual data collection for the project will be conducted by Battelle and Hanford in Richland, Washington.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
There has been considerable discussion of possible genetic and developmental effects of radiation exposure in humans, but our real knowledge of these effects is limited. While animal studies are abundant, human data are virtually restricted to the populations of Hiroshima and Nagasaki, to offspring of women treated with medical irradiation during pregnancy and to vital statistics studies of populations around nuclear facilities. The human data, coupled with the results of experimental studies, suggest the need for population-based studies, particularly at low doses. Studies of populations for which low-level exposure data are available have not been done but are especially needed because of increasing reliance on nuclear power and continuing concern about potential adverse effects. An occupationally exposed population, such as the Hanford workers, with dosimetric data from frequent radiation badge readings and laboratory tests for internal exposures, makes such a study possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43007-01 BB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Peter Flack	Biological Lab Technician	BB	NIEHS
	Susan Smarr	Biologist	BB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry 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 protocols developed for testing model populations. 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. 10-76)

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.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43008-01 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The long term objective of this 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.

(Rev. 10-76)

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 43002-03 BB) and development and use of assays to detect mutagenic substances in body fluids (Z01 ES 43007-01 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-05 BB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	BB	NIEHS
Other:	Charles H. Langley	Research Geneticist	LAG	NIEHS
	Kenneth J. Risko	Mathematical Statistician	BB	NIEHS

COOPERATING UNITS (if any)
Laboratory of Animal Genetics

LAB/BRANCH
Biometry 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 purpose of this work is to develop stochastic models for certain phenomenon arising in population genetics. This work is being done in collaboration with scientists in the Laboratory of Animal Genetics. Current areas of interest are (1) the development of a model appropriate for estimating nucleotide mutation rates from complete DNA sequence data, and (2) the development of a model for studying the population genetics of transposable elements in Mendalizing populations.

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) The model developed earlier to study nucleotide mutation rates from restrictive enzyme data has been modified so that mutation rates can now be estimated from complete DNA sequence data. Approximate formulas for the maximum likelihood estimates have been found and studied via computer simulation. The above work requires knowledge of the ancestral tree of the species under study. Work is now underway to generalize the above work to the case when the ancestral tree is not known. (2) Work is underway to develop diffusion like models for studying the evolution of transposable DNA elements. The difficulty with this problem is that a nonlinear control mechanism must be built into the model so that the number of transposable elements per zygote does not grow indefinitely.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: More realistic models for genetic phenomenon should be beneficial in predicting long-term effects of environmental changes on public health.

PUBLICATIONS

Kaplan, N. and Langley, C.: A new estimate of sequence divergence of mitochondrial DNA using restriction endonuclease mappings. Journal of Molecular Evolution 13: 295-304, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44003-03 BB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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)	BB	NIEHS
Other:	Stephen M. Brown	Medical Officer (Research)	BB	NIEHS
	Anna Bagniewski	Mathematical Statistician	BB	NIEHS
	Beth C. Gladen	Staff Fellow	BB	NIEHS
	Aaron Blair	Staff Fellow	EEB	NCI
	Robert Hoover	Associate Chief	EEB	NCI

COOPERATING UNITS (if any)

Environmental Epidemiology Branch, National Cancer Institute

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 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 is a long-range project designed to improve epidemiologic methods for studying reproductive outcomes, particularly for studying spontaneous abortion, and to investigate the association between environmental exposures and reproductive outcomes. There are several facets to this project. A comprehensive reproductive questionnaire has been developed, and a study has been designed to test the validity of questionnaire data. The basic epidemiology of spontaneous abortion is being investigated using an extensive, previously-unanalyzed data set. Possible confounders in epidemiologic studies of spontaneous abortion are being analyzed, and questions regarding the underlying biological mechanisms of spontaneous abortion are being examined. Finally, a study of reproductive outcomes among workers exposed to perchloroethylene is being prepared. This solvent is widely used in drycleaning and has been shown in the laboratory to be a mutagen and carcinogen.

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 focussed on (1) the development of a comprehensive questionnaire regarding reproductive history and suspected hazardous exposures; (2) a test of the validity of recall data regarding prior spontaneous abortion; (3) a description of the epidemiology of spontaneous abortion, including possible changes in the occurrence of spontaneous abortion over time; (4) an investigation of the association of spontaneous abortion with gravidity; and (5) a study of the possible association of spontaneous abortion with occupational exposure to perchloroethylene, which is a laboratory carcinogen and mutagen.

MAJOR FINDINGS AND PROPOSED COURSE: Most phases of this project have been initiated in this fiscal year and are at various stages of development. (1) The questionnaire has been completed and will be ready for use by the end of this fiscal year. The reproductive questionnaire was prepared under a contract with the Research Triangle Institute, under direct and continuing review by the project officer. This questionnaire has been pilot-tested, approved by the RTI human subjects review board, and submitted for OMB approval. (2) The validity study is under peer review and is scheduled to begin in the early part of the next fiscal year. (3) The first portion of the epidemiologic description of spontaneous abortion is nearly complete, due to the availability of an extensive data set collected over the past forty-five years by Dr. Alan Treloar. The major finding of this analysis has been that, despite major changes in perinatal mortality and changes in presumed exposures to environmental hazards over the past four decades, the occurrence of spontaneous abortion among women in this data set has not changed over time. (4) The analysis of spontaneous abortion by gravidity also uses data from the Treloar study, which offers what may be a unique opportunity to separate the effects of maternal age and gravidity on the risk of spontaneous abortion. A mathematical model is being developed to describe pregnancy outcome, to be tested against the empirical data. (5) The study of drycleaning workers exposed to perchloroethylene is being prepared for peer review. This study is being done in cooperation with the National Cancer Institute, which has already conducted a mortality study of these workers.

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. This project intends to strengthen the epidemiologic tools for measuring such outcomes (particularly spontaneous abortion), and by so doing, to better assess the contribution of environmental factors to human reproductive loss.

COMPARATIVE MEDICINE BRANCH

COMPARATIVE MEDICINE BRANCH
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 microbiologist, one animal husbandman, one secretary, two clerks, fifteen biological laboratory technicians, sixteen biological aids, twelve wage grades and eighteen students.

The addition of the Glassware and Media Kitchen organization to the Comparative Medicine Branch in August 1979 added sixteen people who are composed of eight permanent full-time individuals, three permanent part-times and five stay-in-schoolers. Ms. Juanita Davis is the Supervisor of that unit.

At the present time there are a number of vacancies existing in the Comparative Medicine Branch: one Veterinary Medical Officer; one Veterinary Assistant; one Microbiologist (Virologist); four Biological Aids; three Wage Grades; one Industrial Washer System Operator (PPT); two Laborers (SIS) and two Zoologists (TFT).

Space: Space is the critical concern it was in the past for two reasons. One is that the 6,800 sq. ft. known as the Rodent Breeding Facility which has been contracted for from Duke University where we will be able to maintain the colonies previously held in Building 15 as well as those being received from contracts on rederivation cannot be used until the personnel freeze is lifted to allow personnel to maintain this expansion. The second reason space is so critical is that there is an increase of user personnel without appropriate increase of support personnel.

A contract for space with Becton, Dickinson was terminated at their request as they wish to use the space for their own work.

Programs: The major programs developed and underway during the past year have been curtailed because of lack of personnel and cutback in funds. The development of the program for data system for animal procurement is in operation. Plans are still underway to create data systems for animal census report, equipment, supply inventory, breeding colony information and quality control. However, the time frame has been extended a couple of

years. This was necessary as only one analyst is available, instead of the two previously planned, and equipment funds ($250,000) for computer hardware was withdrawn.

Training programs have further been curtailed because of the reduction and the availability of personnel. There is training continuing on a one-to-one basis in the animal rooms, particularly with the employees assigned to new tasks. The major emphasis is on barrier maintenance where animals have been barrier derived on contract and brought into isolators in our facility.

Programs in quality control have been reevaluated to reduce the numbers of animals per shipment from which necropsies and samples are taken. However, as there has been a dramatic increase in numbers of shipments, the work load in quality control has increased by approximately 20%. The same increase has been documented in work performed in media and glassware with no additional personnel.

Research in veterinary medicine has been curtailed and is much reduced in the quality control laboratory.

One of the programs that appears to be well accomplished this year is the preparation via purchase of equipment for initiating the Comparative Medicine Branch (44 of 68 animal rooms) on the South Campus. Equipment has been purchased ($1,250,000) over a two year period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30008-09 CMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Effects of Environmental Chemicals on Host Resistance to Infectious Agents

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

PI:	J. E. Thigpen	Microbiologist	CMB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	RRP NIEHS
	G. A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section, CMB
Joe Haseman, Ph.D., BB
Histology Section, EBB

LAB/BRANCH
Comparative Medicine Branch

SECTION
Microbiology Laboratory

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.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)

Previous studies have shown that mice exposed to TCDD (5ug/kg body weight) once weekly for 4 weeks, are more susceptible to infection, and that 5-day Thioglycollate induced peritoneal exudates contain significantly less lymphocytes and macrophages than controls. Present investigations are geared to determine whether this reduction in peritoneal cells is due to impaired chemotactic function or due to an antiproliferative effect on pro monocytes or macrophages. Either effect could contribute to increased susceptibility to infection with Salmonella bern.

These studies were performed as a blind study. Experimental data has not been completely summarized; therefore, the identity of the treatment groups will not be revealed until data is completely summarized. Final results will be completed as soon as possible.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: (Blind Study) Four-week old male C57BL/6JFH mice were randomly divided into 2 groups. Each mouse was weighed and dosed via gavage once each week for 4 weeks with TCDD in acetone-corn oil or with only acetone-corn oil. Two days after the last dose of TCDD, a leukocytic exudate was induced in the peritoneal cavity with 2.0 of thioglycollate. Mice were sacrificed at 0, 3, 6, 12, 24, 48 and 120 hours and heart blood, peritoneal exudates, and bone marrow cells were collected for total and differential cell counts. Macrophages were tested for chemotactic function.

MAJOR FINDINGS AND PROPOSED COURSE: These studies have been performed as described above. At the present time, the experimental data has not been completely summarized. Therefore, the identity of the groups have not been revealed. When the data is completed for each group, identity will be revealed and results and findings will be evaluated. These studies will continue when time permits. Service duties have occupied most of my time, therefore little time has been devoted to this project this past year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of environmental chemicals when administered in sublethal doses are immunosuppressive and increased mortality rates have been observed upon exposure to infectious agents. These findings are significant in that humans may be exposed to these same compounds in minute quantities and, therefore, may be rendered more susceptible to certain types of infectious agents. It is important that we establish why mice exposed to TCDD are more susceptible to bacterial infections.

ENVIRONMENTAL BIOLOGY BRANCH

ENVIRONMENTAL BIOLOGY BRANCH
Summary Statement

The Environmental Biology Branch assists and collaborates with NIEHS laboratories and research programs in the fields of inhalation toxicology, pathology, electron microscopy and histology; plans, conducts and collaborates in basic and applied studies in these fields; and plans, conducts and coordinates toxicologic bioassays on selected environmental chemicals.

The comparative toxicity of halogenated polyaromatic hydrocarbons, a principle focus of activity for several years, continued with specific chlorinated dibenzofuran and brominated naphthalene isomers being of current interest. The isomers to be studied are first determined based on predicted toxicologic properties and synthesized by the Environmental Chemistry Branch. The toxicity data further supports the previously formed hypothesis that toxicity is dependent on degree and specific location of halogenation of the aromatic ring and that the toxicity observed is a general syndrome suggesting a common mechanism of action.

Toxicopathologic studies of polybrominated biphenyl (PBB) focused on the chronic effect of a six-month exposure in rats and mice. The animals were sacrificed at the end of six-month treatments and the following parameters were studied: food consumption, body weight gain, hematology, clinical pathology, anatomic pathology and tissue porphyrins. Animals from each dose group are being held for life-time observation. Preliminary examination of the data indicates a remarkably high incidence of hepatic neoplasm in exposed rats compared with control. The effectiveness of charcoal, cholestyramine, and caloric restriction in reducing the body burden of PBB was also studied. None of these methods significantly enhanced PBB excretions.

In collaboration with the Laboratory of Behavioral and Neurological Toxicology, a series of experiments were initiated with Kepone in rats to establish the dose range in which fertility, reproduction or neurobehavioral effects are observed. Neurobehavioral effects were observed at doses below those which affected fertility and reproduction. Further experiments are in progress to more precisely characterize these toxic effects, determine their duration, and to determine if progeny of Kepone exposed females are at risk.

Several major long-term projects progressed according to schedule:

1. The three-species vinyl chloride study which seeks to assess the functions of age and duration of exposure upon carcinogenic response will be completed by the end of FY '80. The results in rats show that there is a dose response relationship in the increase of angiosarcomas with increasing exposure durations. Age at the time of exposure also seems to be a critical factor in incidence of angiosarcomas in rat. Hepatocellular carcinomas and mammary gland carcinomas were also increased. In hamsters, the highest incidence (15%) of angiosarcomas were found in hamsters exposed 0-6 months to vinyl chloride. Increasing exposure duration, or beginning later

in life, results in lower incidence of angiosarcomas. Mammary gland carcinomas were also produced in hamsters by 0-6 months or longer exposure of vinyl chloride. The tissues from mice are still being evaluated.

2. The rat inhalation study which seeks to compare the fibrogenic and neoplastic response of chrysotile asbestos that varies as to fiber length and of a fibrous glass is currently in progress. Early results clearly indicate a difference in severity of lung fibrosis dependent upon the length of the chrysotile fiber. Gross lesions suggestive of pulmonary tumors have been found in 5, 6 and 7 animals exposed to short, intermediate and chrysotile B fibers respectively.

3. The oral asbestos studies in the hamster are currently in the pathology findings review phase; rat studies are in their eighteenth month with minimal mortality observed to date. Chrysotile, amosite, and crocidolite asbestos and tremolite are being evaluated through this contract.

4. To determine the fetal toxicity of pentachloroanisol (PCA), pregnant mice were treated orally on day 6 through 15 of gestation period. Fetuses were obtained by caesarean section on day 18 of gestation and examined histologically. The study is in progress.

Responsibility for the Environmental Teratology Information Center (ETIC) was transferred to the Branch late in FY 1978. Since July, 1975, ETIC has built a computer file of approximately 15,000 records and assembled a document library that contains every paper indexed. Each citation has been indexed with all bibliographic information, common and taxonomic name of test object, and Chemical Abstract Service (CAS) Registry Number. Titles may be searched using key words from the title.

The Chemical Agent Registry has been updated to include chemical name fragments and Wiswesser Linear Notations (WLN). As a result, chemicals in the ETIC Agent Registry may now be searched by synonym, CAS number, fragment as part of a chemical name, molecular formula, complete structure or structural fragment by diagram, and functional or major chemical components by name (e.g., esters, ethers, epoxides, etc.).

The ETIC file is now one of the subfiles of the National Library of Medicine's TOXLINE (Toxicology Information Online) and is available through a nationwide NLM network of centers at more than 900 universities, medical schools, hospitals, Government agencies, and commercial organizations as well as a number of foreign centers.

The ETIC file is also one of the data bases provided by RECON (Remote Console), a remote-access computerized storage and retrieval system located at Oak Ridge National Laboratories and available to all Department of Energy divisions and contractors.

The Chemical Agent Registry has been made a part of the Environmental Protection Agency/National Institutes of Health Substructure Search System and thereby offers still another search capability.

An average of 33 inquiries a month requesting information on over 100 agents and almost every test object are received and processed directly by the ETIC staff. There is no way to determine the number of requests processed through TOXLINE and RECON since they do not monitor searches on those systems.

It is proposed that ETIC will continue to collect and process papers from current and previous years and provide regular updates of the ETIC/TOXLINE subfile and the ETIC/RECON data base. We hope to be able to add author abstracts to the file. The author's address is one new field to be added and is already a part of a number of the other files on TOXLINE. You will be able to identify experts in a particular area by searching with zip codes. Based upon the needs and requirements shown through reviewing search requests, other new files may also be added. Publications of special search subjects will be prepared and made available upon request. A bibliographic listing will be prepared and updated on a regular basis. Primary effort will go into the next major undertaking of ETIC. They will begin the extraction of more detailed information from each reference. This information will then be entered into a Teratology Data Bank.

The Comparative Pathology Section: maintains a centralized NIEHS service facility for electron microscopy and histology; provides a diagnostic service for lesions observed in laboratory animals; provides a hematology and clinical chemistry service; and plans, conducts and collaborates with NIEHS research programs in research activities utilizing various techniques of experimental pathology.

The Section supported the disease surveillance program of the Comparative Medicine Branch by conducting necropsies and histopathologic interpretation of 781 cases during the year. It also provide histologic, EM or pathology support to 27 projects within eight intramural research laboratories. Sixteen independent or collaborative studies were supported within EBB. This Section is also actively engaged in supporting various projects within the National Toxicology Program. The independent research conducted in this section includes: (1) assessment of bone marrow toxicity using *in vivo* and *in vitro* culture systems for hemopoietic progenitor cells. The effect of PBB, TCDD, DES, Indomethacin Fryol FR-2 and benzpyrene is being studied. This project is currently under progress) and (2) a comparative toxicity study of technical vs. pure Pentachlorophenol (PCP) in Holstein cattle was undertaken and it was found that technical PCP is more toxic than pure PCP.

The program of the Inhalation Toxicology Section is composed of two parts: (1) Studies are conducted of compounds to which toxicologically significant exposure would be expected to be primarily by inhalation. Research is focused on expressions of toxicity at the levels of tissues, organs, and organ systems, with emphasis on the cardiovascular system and (2) A principle effort of the Section is directed toward the advancement of inhalation toxicology. The facility is designed for exposure of small laboratory animals to organic vapors, cylinder gases, and oxides of nitrogen.

The small animal inhalation facility is now operational under machine control. Data acquisition and chamber control are by means of an advanced electronic calculator rather than a minicomputer which, although somewhat slower and less versatile than a "mini", serves the job reasonably well. The system controls 6 pairs of chambers, but is designed to accommodate up to 9. As more chemical analytical instruments are acquired, more pairs of chambers may be operated, or multiple compounds may be run in the same chamber(s). The compounds of interest in the chamber are monitored with infrared gas analyzers whenever possible, but provision has been made for the use of gas chromatographs as well. Data acquisition and control are more efficient when the infrared analyzers are used.

The Section research program is divided into five parts. They are studies of environmental contaminants on (1) the electromechanical activity of isolated, perfused whole hearts and other myocardial preparations in vitro; (2) the composition and function of pulmonary lavage and its constituents; (3) pulmonary function in small animals; and (4) a new study to test the hypothesis that exposure of laboratory animals to NO_2 during concurrent administration of heterocyclic amines leads to the formation in vivo of nitrosamines that result in tumor formation. A fifth project serves as an umbrella for miscellaneous, related studies and is called Toxicology of Environmental Chemicals. Isolated perfused rabbit hearts and other heart preparations are being used for the purpose of evaluating the effects of allylamine on myocardial function.

The Biochemical Toxicology Group has completed studies on the structure-activity relationships for induction by halogenated biphenyls. Results thus far indicate that compounds which are isosteric with 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD) are more toxic and interact with the receptor for induction of AHH. A number of halogenated benzenes have been studied as inducers of hepatic enzymes. Of these, only hexachlorobenzene is a "mixed inducer" with inductive properties intermediate between those of TCDD and phenobarbital. This correlates with certain toxicities produced by HCB, but not other chlorinated benzenes. Gel electrophoretic and purification work is proceeding to identify the cytochrome induced by this compound. A number of renal function tests have been evaluated as indicators of nephrotoxicity. These indicate that many standard tests of renal function are not very sensitive. The most sensitive tests were water deprivation and organic ion concentration in vitro. Other in vitro assays indicate the possible usefulness of a completely in vitro approach. The results of these studies will be used to suggest renal tests for the National Toxicology Program. A study with 1,2-dibromo-3-chloropropane (DBCP) has been initiated. Both the renal and testicular lesions are being examined physiologically and morphologically. DBCP affects the proximal tubule of the kidney, and inhibits spermatogenesis. The pharmacokinetics of DBCP are being examined, and hopefully metabolite formation and disposition can be related to the site and severity of the lesions.

The Immunology Work Group is studying to clarify differences among several methods for measuring the same immunological parameter(s) for assessing immunotoxicity and altered host resistance in order to define a testing battery. The group is presently developing, refining and applying methods for

measuring delayed hypersensitivity as well as T-lymphocytes, B-lymphocytes, macrophages and bone marrow cell function 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. These studies will hopefully allow correlations between changes in immunological parameters to provide prognosticators of alterations in host resistance to bacterial and viral diseases or tumors. Investigation of the immunological and toxicological effects of PBB in Michigan Farmers and chemical workers is being studied through a contract. The Michigan and Wisconsin population to be studied have been contacted and logistics have been developed for the pending visits and evaluation of these individuals.

The Chemical Disposition Group is studying the metabolism and disposition of halogenated alkyl phosphates and pharmacokinetics of chlorinated xenobiotics. 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 of 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.

The primary long-term goal of pharmacokinetics of chlorinated xenobiotics is 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 for simple halogenated aromatics the rate of metabolism is limited by the availability of adjacent unsubstituted carbon atoms which are thought to facilitate metabolism via arene oxide intermediates. This work has also established that halogenated aromatics are readily absorbed from the gastrointestinal tract, that those compounds which are not 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. This work has demonstrated that acute as well as chronic toxicity may be related to the exposed animals ability to metabolize and excrete the toxic compound, and that for tetrachlorodibenzofuran (TCDF) chronic toxicity may not be manifest prior to the accumulation of a critical body burden.

A commitment of Branch scientists is to the recently established National Toxicology Program. In addition to laboratory research, scientists regularly participate in experimental design and subsequent interpretation of lifetime rodent bioassays. The EBB scientists are also monitoring two major research contracts under the NTP program. These are: (1) 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; and (2) metabolism, distribution and excretion of selected xenobiotics which are of particular interest to NTP or intramural scientists of the NIEHS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30038-03 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Characterization of Cytochrome(s) P-450 Induced by Hexachlorobenzene

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

PI:	J.A. Goldstein	Pharmacologist	EBB NIEHS
OTHER:	P. Linko	Chemist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology 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)

This study was initiated to determine whether the effects of hexachlorobenzene (HCB) on drug metabolism and on toxicity are related to contamination by chlorinated dibenzodioxins or other impurities, or whether HCB acts through a mechanism similar to the dioxins. This work was extended to determine whether the cytochrome(s) P-450 induced by HCB were identical to that induced by 3-methylcholanthrene (3-MC) and 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats and 3-MC susceptible and resistant strains of mice were dosed orally with HCB and other chlorinated benzenes. Cytochromes were characterized by substrate specificities and by gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Rats exposed to HCB show and intermediate pattern of induction similar to a mixture of phenobarbital and 3-MC. However, induction of cytochrome P-448 dependent enzymes was less than was seen with 3-MC. Gel electrophoresis indicated increases in three proteins in the 50-60,000 region, including two proteins increased by phenobarbital and one of the two proteins increased by 3-MC. HCB increased the protein which probably corresponds to cytochrome P-448, but not P_1-450, while 3-MC increased both P-448 and P_1-450. A number of other di, tri, tetra- and pentachlorobenzenes were tested, and in contrast to HCB, were found to be pure phenobarbital-type inducers. HCB induced cytochrome P-448 dependent enzymes in 3-MC responsive, but not in nonresponsive mice.

We will attempt to purify enzymes from 3-MC, phenobarbital, and HCB-induced animals and compare these cytochromes in more detail. We are also purifying HCB using a charcoal column to remove possible planar impurities and will retest to determine whether HCB or impurities are responsible for these effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: HCB is a widespread environmental contaminant. HCB resembles the more toxic dioxins in some of its effects. These compounds are quite different structurally. It is important to know whether the HCB and dioxins operate through the same mechanism and to be able to predict the structure-activity relationship since toxicity and induction appear to be related.

PUBLICATIONS

Goldstein, J. A. and Linko, P. Alteration of hepatic cytochrome P-450s by chlorinated benzenes (CBs). Fed. Proc. 39, 864 (Abstr.) (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30042-05 EBB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	EBB NIEHS
	M. P. Moorman	Biomedical Engineer	EBB NIEHS
	R. Sloane	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

Pathology Section, EBB

LAB/BRANCH

Environmental Biology Branch

SECTION

Inhalation Toxicology Section

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. 10-76)

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. (Ed.): Proceedings, Target Organ Toxicity: Cardiovascular System. Environ. Health Persp. 26: 148-285, 1978.

Van Stee, E.W.: Autonomic innervation of the heart. Environ. Health Persp. 26: 151-158, 1978.

Van Stee, E.W.: Myocardial Toxicity. In Witschi, H (Ed.): The Scientific Basis of Toxicity Assessment. Amsterdam, Elsevier/North-Holland, pp. 167-182, 1980.

Van Stee, E.W. (Ed.): Cardiovascular Toxicology. New York, Raven Press, 1981.

Van Stee, E.W. : Overview of cardiovascular toxicology. 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30044-04 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
OTHER:	M.P. Moorman	Biomedical Engineer	EBB NIEHS
	P.C. Wynns	Biological Aid	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.1	0.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A. Morpholine is excreted in the urine and bile following exposure by any route. The chemical might be expected to be excreted via the renal tubular base transport system, but evidence indicates that this may not be so. Furthermore, although its lipid solubility may be controlled through regulation of urinary pH, its rate of elimination in the urine is not. Studies are continuing to elucidate the renal disposition of this chemical.

B. Preliminary studies indicate that time-varying concentration profiles may affect expressions of inhalation toxicity. Rats exposed to a variety of profiles with equal concentration times time, exhibit different degrees of chemical injury to the liver by carbon tetrachloride.

METHODS EMPLOYED: A. Rabbit renal cortical slices are incubated in Dulbecco's modified Eagle's medium or Daniel's Minimum Essential Medium. Isolated kidneys are perfused in an apparatus of standard general design. Clearances are determined by conventional methods. Morpholine labeled uniformly with ^{14}C, TEA labeled with ^{3}H, and PAH labeled with ^{14}C are used in the studies. Oxygen consumption of tissues is determined using an oxygraph. Cold chemicals of particular interest used are morpholine, 2-aminoethoxyethanol, diethanolamine, 2,6-dimethylmorpholine, TEA and PAH. B. Male Fisher rats, 100-150 g are exposed to air or various time-varying concentration profiles of CCl_4 that all have a concentration times time equal to 1500 ppm-hr. Serum sorbitol dehydrogenase activities and histopathological evaluation of the livers are used as indexes of hepatoxicity.

MAJOR FINDINGS AND PROPOSED COURSE: A. A variety of in vivo and in vitro experiments was conducted from which the conclusion was reached that the primary route of the elimination of morpholine was via the kidneys. The concentration of ^{14}C-morpholine in the renal cortex of rabbits was 6.6 times the blood concentration, and in the renal medulla 15.3 times the blood concentration following intravenous bolus injections. Morpholine did not affect the uptake by renal cortical slices of tetraethylammonium (TEA) and the rate of the urine eliminated was unaffected by urinary pH. The conclusion was reached that morpholine is transported by the renal tubules by a rate-limited mechanism distinct from the organic base transport system responsible for the transport of TEA. B. Preliminary studies indicate that differences exist among the treatment groups. Since each group is exposed to the same C x T, the variations in degree of toxic injury to the liver must be attributable to the exposure profile. The ultimate objective will be to determine what features of the exposure profile contribute to the expression of toxicity and to what degree. Variables like C x T, number of leading edges, maximum concentration, etc, will be analyzed using a multiple regression approach.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Little is known, in general, regarding the specific renal pharmacology of heterocyclic amines. The amines are of general toxicological significance because of their potential for conversion to nitrosamines known to be significant animal carcinogens. A comprehensive approach to nitrosamine toxicology requires the inclusion of studies of the precursors, when such information is not otherwise available. Only through an understanding of the contributions of the individual parts of a hypothetical scheme can one hope to appreciate the significance of the in vivo interactions that may lead to the final endpoint which, in this case, is the expression of nitrosamine toxicity in an animal model.

SUMMARY: A. Morpholine and related amines are subject to conversion to the corresponding nitrosamines by nitrite at acid pH and by NO_2 (N_2O_4) at neutral and alkaline pH. The nitrosamines may be significant animal carcinogens. Thus, the potential exists for chemical interactions that would lead to the formation in vivo of nitrosamines. A complete understanding

of the whole process requires that information be acquired not only in regard to the proximate toxicant (nitrosamine) but also the precursors (e.g. heterocyclic amines). The kidney appears to be a significant portal of excretion for heterocyclic amines and, as such, a goal of this research is the elucidation of the renal pharmacology of the amines using morpholine as the principal model compound. Computerized inhalation facility operation has removed a barrier to the reliable reproduction of time-varying exposure profiles in the inhalation toxicology laboratory.
B. Experiments in inhalation toxicology traditionally have centered around non-time-varying exposure profiles in which chamber concentrations were rapidly raised to some predetermined point, held there for a specified period, and then reduced to zero as rapidly as possible. This may not always be the best model for the system under study. Our preliminary results indicate that when the traditional approach does not accurately represent the system under investigation, the inferences may be erroneous.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30045-03 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
	E. W. Van Stee	Physiologist	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

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. 10-76)

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30075-03 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Comparative Toxicity of Technical vs Pure Pentachlorophenol in Dairy Cattle

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	EBB NIEHS
	C.E. Parker	Research Chemist	LEC NIEHS
OTHER:	J.A. Moore	Senior Veterinary Medical Officer	EBB NIEHS
	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	M.I. Luster	Research Chemist	LEC NIEHS
	J.A. Goldstein	Research Pharmacologist	EBB NIEHS
	A.H. Rakes	Animal Scientist	NCSU
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS
	F.A. Talley	Histology Technician	EBB NIEHS

COOPERATING UNITS (if any)

Animal Science Department, North Carolina State University, Raleigh, North Carolina

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this 160 day feeding study was to determine the effects of unwanted contaminants in technical pentachlorophenol (PCP) in dairy cattle by comparing them to pure PCP and non-exposed animals. Parameters studied included clinical examination, weight gain, hoof growth, hematology and clinical chemistry, gross and histopathology, electron microscopy, immunology, hepatic enzyme induction, and chemical analysis of tissues. Results show that technical PCP is significantly more toxic than pure PCP.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this study was to compare the effects of technical (t) vs analytical (a) grade PCP in cattle. Four groups of 3 female yearling Holstein calves were exposed for 160 days via feed to aPCP or tPCP and mixtures thereof. A fifth group served as unexposed controls. All treated animals received the same amount of PCP; 20 mg/kg/day for 42 days followed by 15 mg/kg/day. Fat biopsies for chemical analyses were obtained before, during (70 days) and at the end of the study. Liver samples were collected at the latter two times. Parameters studied included clinical signs, feed consumption, weight gain, hoof growth, hematology and clinical chemistry, urinalysis and immunology. At the termination of the experiment the animals were necropsied and representative tissues were collected for histopathology, electron microscopy, hepatic enzyme induction evaluation, immunology, and chemical analysis [see LEC (Parker) for details].

MAJOR FINDINGS AND PROPOSED COURSE: Results show that technical PCP is significantly more toxic than pure PCP. Major findings included a dose related decrease in body weight gain, decreased feed efficiency and progressive anemia in tPCP cattle while those exposed to aPCP were comparable to the untreated controls. Liver and lung weights were increased while the thymus was decreased. Marked villous hyperplasia of the urinary bladder was found in 2 of 3 animals exposed to the highest level of tPCP. There were minimal hepatic lesions. Immunological studies suggested a progressive dose related alteration in cell mediated immunity but no effect on humoral immunity. Liver mixed function oxidases were increased moderately by aPCP, but markedly by tPCP. This study indicates that the toxicity of PCP in cattle is primarily attributable to its contamination with toxic impurities. Project is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pentachlorophenol is widely used as a wood preservative and fungistatic agent. In some cases it is contaminated with unwanted toxic chemicals such as dibenzo-p-dioxins. There have been clinical situations in which PCP treated wood has been incriminated as a etiologic factor in diseased dairy cattle in the north central United States. Because of this farm problem and the potential of contaminated food (beef) reaching humans, it is important to assess the toxicity of PCP in dairy cattle and to determine the importance of contaminants therein.

PUBLICATIONS

McConnell, E.E., Mcore, J.A., Gupta, B.N., Rakes, A.H., Luster, M.I., Goldstein J.A., Haseman, J.K., and Parker, C.E. The chronic toxicity of technical and analytical pentachlorophenol in cattle. I. Clinicopathology. Toxicol. Appl. Pharmacol. 52: 468-490, 1980.

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 analysis of tissues. Toxicol. Appl. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30084-03 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Dose-response of Drug-metabolizing Enzymes to Halogenated Biphenyls and Napthalenes

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

PI:	J. A. Goldstein	Pharmacologist	EBB NIEHS
OTHER:	P. Linko	Chemist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	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)

The polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) are inducers of hepatic mixed-function oxidases. The purpose of these studies is to determine the structure-activity relationships of PCB and PBB isomers and other components of the commercial PBB and PCB mixtures, as inducers of cytochromes P-450 and P-448 and to relate these effects to toxicity.

FS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS: Results thus far are consistent with the hypothesis that planarity and halogenation of the 3,4,5 and 3',4',5'-positions on the biphenyl rings are important for induction of AHH and that induction and toxicity are the result of interaction with a common receptor. Naphthalenes appear to be less potent because they do not meet the 3 x 10 A° size requirement.

A chapter reviewing the biochemical effects of biphenyls, naphthalenes, dibenzodioxins, terphenyls and related compounds has been completed and submitted for publication.

PROPOSED COURSE: Three brominated naphthalenes will be compared in the chick embryo versus the commercial Firemaster BP6 for potency, since metabolism will be more or less ruled out in this system, to determine whether these compounds have more intrinsic potency than is evident from work in the rat where metabolism could complicate the results. The cytochromes induced by these compounds are being partially purified to be compared with the phenobarbital and 3-methylcholanthrene induced cytochromes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There has been considerable human exposure to PBBs and PCBs (polychlorinated biphenyls). These experiments were designed to analyze the structure-activity relationships of PCBs and PBBs. Toxicity appears to correlate well with structure-activity relationships. In particular, those compounds which induce P-448 are the most toxic.

PUBLICATIONS

Goldstein, J. A. The structure-activity relationships of halogenated biphenyls as enzyme inducers. N. Y. Acad. Sci., 320, 164-178, 1979.

Goldstein, J. A., Linko, P. C., Levy, L. A., McKinney, J. D., Gupta, B. N. and Moore, J. A. A comparison of a commercial polybrominated biphenyl mixture 2,4,5,2',4',5'-hexabromobiphenyl and 2,3,6,7-tetrabromonaphthalene as inducers of liver microsomal drug metabolizing enzymes. Biochem. Pharmacol. 28, 2947-2956, 1979.

Book Chapter

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, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30085-03 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Early Detection of Lung Injury

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	EBB NIEHS
	E. W. Van Stee	Physiologist	EBB NIEHS
OTHER:	W. Gibson	Chemist	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

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 development of toxicity indexes based on lung lavages requires the exposure of a large number of animals to inhalation of toxic substances. Part of the effort can be reduced by running preliminary tests in vitro using cells derived from the target organ such as alveolar macrophages. Since alveolar macrophages remain differentiated and continue to express cell specific traits during short term culture in vitro, they are particularly suited for the study of the effects of toxic substances on their specific properties. The effects of morpholine were studied on alveolar macrophages in vitro. The effects observed not only agreed with those observed in vivo, but they also gave indications on the mechanisms involved.

PHS-6040
(Rev. 10-76)

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 and 31 mg penicillin and 50 mg streptomycin). The final cell concentration was 1.5×10^6 to 2×10^6 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. In our first experiments the test material was morpholine and the reuslts were used to substantiate our in vivo experiments. The concentration of morpholine used was 2.3 mM, the incubation period 20 hrs and the atmosphere of incubation contained 5% CO_2. At the end of the incubation period the cells were taken up using Trypsin-EDTA solution. The collected macrophages were sonicated for enzyme analysis. The questions asked in the first experiments were: (1) Do the in vitro experiments agree with the previous in vivo results? (2) Is the increased enzyme synthesis due to new protein synthesis or enzyme activation? (3) Do the increased enzyme activities in the presence of morpholine persist after morpholine is removed from the incubation medium? (4) Does the increase in enzyme activity occur in the same subcellular compartment with which the enzymes are normally associated?

MAJOR FINDINGS AND PROPOSED COURSE: The following enzyme activities were measured: α-mannosidase, β-N-acetylglucosaminidase, acid phosphatase, and 5'-nucleotidase. The measurement of hydrolases was chosen because of (1) the potential capacity of the lysosomal hydrolases to cause tissue damage and inflammation and (2) because these enzymes were used in our in vivo experiments. 5'-Nucleotidase was used as an index of membrane integrity.

It was found when morpholine was present that the increases of α-mannosidase and acid phosphatase were highly significant ($P<.01$) and that of β-N-acetylglucosaminidase was increased ($P<.05$) only in macrophages derived from females. The binding of concanavalin-A was increased in macrophages exposed to morpholine. In contrast the 5'-nucleotidase activity decreased ($P<.05$) in the macrophages exposed to morpholine.

Macrophages were incubated for 20 hr in the presence of morpholine, after which the medium was removed and the cells were washed and reincubated for an additional 40 hr in the absence of morpholine. The macrophages that had been exposed previously retained their high enzyme activities as compared to those not previously exposed to morpholine.

The increase in enzyme activity agrees with the previously reported results from the in vitro experiments. The persistance of the elevated enzyme levels in vitro after 40 hr, in contrast to the in vivo experiments, may be the result of a renewal of the macrophage population in vivo, part of which had not been exposed to morpholine.

The decreased activity of 5'-nucleotidase and the increased activity of Concanavalin-A binding indicate that some alteration in plasma membranes

occured as a result of morpholine exposure.

When lung macrophages were exposed to morpholine in the presence of cycloheximide no increase in enzyme activities was observed as compared to controls plus cycloheximide thus indicating that the increased activity in the presence of morpholine was due to new protein synthesis.

The increases of all of the enzyme activities that were measured were of the same magnitude regardless if they were expressed as per cell or as per mg of protein. Subfractionation of cultured lung macrophages indicated that the increase of enzyme activity occured in the subcellular compartment with which the enzyme was normally associated. Thus the intracellular distribution of enzymes remained the same for controls and those exposed to morpholine.

The studies reported here indicate that the in vitro experiments can be used not only to substantiate the in vivo experiments but also to achieve a higher degree of experimental control over the populations of cells under study.

The use of in vitro tests as a major effort requires the development of well defined biochemical media and culture conditions, and the study of critical cell properties as a function of incubation time and other variables.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A great need exists for the development of indices of early inhalation toxicity. Lung macrophages are among the first lung cells coming into contact with inhaled toxic materials. Lung macrophages provide an early pulmonary defense mechanism and as such, alterations in their function could be reflected in consequences of altered pulmonary defense. Furthermore, the macrophages contain enzymes that could participate in the biotransformation of xenobiotics resulting in either activation or detoxification with consequences for the lung.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30089-02 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
OTHER:	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Chemistry Laboratory, NIEHS

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The object of this study was to determine if activated charcoal (AC) or cholestyrimine (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.

PHS-6040
(Rev. 10-76)

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 cholestyrimine 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 cholestyrimine
IV	"	"	"	"	and periods of restricted food intake
V	FF-1	"	"	"	
VI	"	"	"	"	and charcoal
VII	"	"	"	"	" " and restricted calories
VIII	"	"	"	"	and cholestyrimine
IX	"	"	"	"	" " and restricted calories

After six 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 cholestyrimine 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30091-02 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

A Comparison of Low Level Exposure of Polybrominated Biphenyls (Firemaster FF-1) in the Diet vs Gavage in Male CDF Rats

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

PI:	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Biology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1		0.1 technical

CHECK 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 study in male CDF rats was to compre the effects of a low level of polybrominated biphenyls (Firemaster FF-1) administered in the diet with the same level via gavage. In addition, to compare the rate of body weight gain in nonexposed rats given powdered diet versus the same diet in a pelleted form.

PROJECT DESCRIPTION

METHODS EMPLOYED: Male CDF rats, 6-7 weeks of age were fed ground NIH rodent chow containing Firemaster FF-1 at 1 mg/kg/day. Food consumption was measured 3x weekly; body weight changes were measured once weekly. The control rats being fed either ground or pelleted chow were handled in the same manner. All rats were fed ad libitum for 6 months.

MAJOR FINDINGS AND PROPOSED COURSE: There was no significant difference in the effects of FF-1 between the two routes of exposure in the parameters measured. Additionally there was no difference between the control animals fed ground diet compared to pelleted diet. This project is completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Administration of test substances to a large group of animals via the diet requires less technical time than does daily gavage. In long-term chronic exposure studies this is of importance. The relative ease of adding test substances to ground feed as opposed to pelleting after the addition of test substances is also of great time-saving value in toxicological testing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30098-02 EBB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Tumorigenic 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	NIEHS EBB
	G.A. Boorman	Veterinary Pathologist	NIEHS EBB

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

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)

Random bred, male Fisher 344 rats, CD-1 mice, and golden Syrian hamsters were exposed to 1-2 ppm of NO_2 for 6 hr/da, 5 da/wk for 30 weeks. Some of the animals also received 0.1% morpholine in deionized water as their sole source of drinking water. The animals will be held for 2 years or until death during which time all animals will receive deionized water. Animals will be killed for histopathological evaluation following the postexposure holding period.

PHS-6040
(Rev. 10-76)

METHODS EMPLOYED: Rats, mice, or hamsters were exposed to NO_2 (N_2O_4) in dynamic, flow-through chambers. Exposures were conducted for 6 hr/da, 5 da/wk, for 30 weeks. Subgroups of animals were given morpholine in the drinking water. Animals are to be killed approximately 2 yr from the beginning of the exposures for gross and histopathologcal examination.

MAJOR FINDINGS AND PROPOSED COURSE: Male CDF rats, CD-1 mice, and golden Syrian hamsters were divided into 4 groups each of 35, 35, and 40 animals, respectively. One group of each species was exposed to air and received deionized water as their sole source of drinking water. Other groups were exposed to 1-2 ppm NO_2 and received plain water. The third groups were exposed to air and received 0.1% morpholine as their sole source of drinking water. The last groups were exposed to the NO_2 and also were given the morpholine drinking water. All of the mice have either died or been killed. Deaths occurred sporadically throughout the postexposure period but were concentrated during the period of 12-14 months post-exposure. Histopathological results are not yet available. Some rats and hamsters have died as the result of expected attrition throughout the postexposure period. They will be killed for examination when they reach approximately 2 years of age. A British group demonstrated in vitro that extensive nitrosation of morpholine by NO_2 took place in plasma. An Illinois group recently demonstrated the presence of N-nitrosomorpholine in whole mouse powders derived from animals gavaged with morpholine and acutely exposed to NO_2 by inhalation. Another British group demonstrated that N-nitrosomorpholine was activated to a proximate carcinogen by rat liver microsomes. A Philadelphia group demonstrated single and double stranded breaks in nucleic acids derived from rats exposed to N-nitrosomorpholine. Many groups have demonstrated the in vivo carcinogenic potential of N-nitrosomorpholine. An NIEHS group has demonstrated that a P450 monooxygenase system capable of mediating the activation of N-nitrosomorpholine is concentrated in bronchiolar Clara cells, cells that have been suggested by an NCI group to be a target for N-nitrosomorpholine carcinogenesis. The proposal is made to prepare whole lung homogenates and/or fractions thereof, to which heterocyclic amines will be added followed by bubbling with NO_2. The formation of N-nitrosomorpholine and its beta-oxidation product will be evaluated by physical analysis and in vitro bioassay.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE ISTITUTE: Determination of the quality, quantity, and distribution of tumors in the animals that already have been exposed, coupled with what has been reported regarding the in vivo formation and disposition of nitrosamines will shed light on a potentially important aspect of carcinogenesis as it may be related to our chemical environment. This is the first report of experiments designed to define a possible chemical link between exposure to a significant urban air pollutant and carcinogenesis in a laboratory animal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30099-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
OTHER:	K. Kohli	Visiting Associate	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this work is to determine the effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and certain polychlorinated biphenyls on arachidonic acid metabolism.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: This project has been initiated in late 1980. The first step will be to ascertain whether TCDD, 3,4,5,3',4',5'-hexachlorobiphenyl or 2,4,5,2',4',5'-hexachlorobiphenyl alters total C^{14}-arachidonic metabolism or the profile of metabolites in tissues in which metabolism can be measured (lung, kidney).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There has been considerable interest in human exposure to TCDD and PCBs. Certain isomers are more toxic than other isomers. It is of interest to determine what biochemical alterations are specific to the more toxic isomers and may be involved in toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30100-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Toxic Effects of 1,2-Dibromo-3-Chloropropane on the Urogenital System in Rats

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

PI:	William M. Kluwe	Staff Fellow	EBB NIEHS
OTHER:	Insu P. Lee	Toxicologist	LRDT NIEHS
	Frank Harrington	Biological Laboratory Technician	EBB NIEHS
	Ralph Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

Laboratory of Reproductive and Developmental Toxicology, NIEHS

LAB/BRANCH
Environmental Biology Branch

SECTION
Biochemical Toxicology

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)

Male pesticide formulators exposed to low concentrations (less than 1 ppm in air) of the nematocide 1,2-dibromo-3-chloropropane (DBCP) exhibit lowered sperm counts and, in some cases, complete aspermatogenesis in testicular biopsies. This project is designed to reproduce the urogenital DBCP lesions in rats and to study factors such as dose-response, cumulative effects, mechanism of toxicity and reversibility of the lesion. The distribution and disposition of ingested, inhaled and cutaneously absorbed DBCP will be determined to assess the hazards of different routes of exposure. Effects on the urogenital tract of other halogenated hydrocarbon compounds reported to produce germinal aplasia (e.g., 1,2-dibromoehane, tris [2,3-dibromopropyl] phosphate) will be compared to those of DBCP.

FS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: The primary objectives of this project are to elucidate the mechanism of DBCP-induced aspermatogenesis, ascertain the reversibility of the testicular lesion, and assess the hazard of mammaliam exposure to low concentrations of DBCP and structurally-related halogenated chemicals. Secondary objectives are to determine the pharmacokinetics of DBCP in rats and the mechanisms of extratesticular DBCP toxicities such as gastric oncogenicity and acute kidney and liver injury. In addition, attempts will be made to improve upon the methodologies currently employed to detect and characterize sperm abnormalities and testicular degeneration in routine toxicity studies.

METHODS EMPLOYED: The pharmacokinetics of DBCP in male rats will be determined using a high dose (acutely toxic), a very low dose and several doses in between if the pharmacokinetics are found to be dose-dependent. Testicular effects will be monitored by direct histological examination of the testis, morphological evaluations of epididymal and tubular spermatogonia, spermatocytes and spermatids, density of epididymal sperm and gross testicular weight. Comparisons will be made between the effects of acute (single dose) and subchronic (repeated doses) treatments with DBCP.

Toxic effects of DBCP on the kidney, liver and stomach will be monitored by histological examination of the tissues and by selected organ function tests.

MAJOR FINDINGS AND PROPOSED COURSE: A single exposure to a sublethal dose of DBCP has produced massive kidney injury and damage of lesser severity to the liver. Morphologically, the renal lesion is initially localized in the S_3 segment (pars recta) of the proximal tubule, but progresses, with time, to include the more distal portions of the nephron, as well. Functionally, tubular reabsorptive processes appear to be impaired.

Rats treated once with a sublethal dose of DBCP exhibited lower testicular weight-to-brain weight ratios than did controls, indicating testicular atrophy or degeneration. This effect was noted 24 hr after treatment and was still evident 10 days later.

In addition to inhibiting spermatogenesis, DBCP has been shown to cause mutations (dominant lethal) in rats. Therefore, an evaluation of the effects of DBCP treatment of paternal rats on the formation and development of offspring may be conducted. Also, the toxic effects of DBCP on developing animals (both *in utero* and post-birth) will be compared to those in adult rats since the deleterious effects of ionizing radiation, including germinal aplasia similar to that produced by DBCP, are known to be greater in immature rats than in adults.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
DBCP toxicity - germinal aplasia - has been documented in humans. This project provides an animal model for the effects observed in humans and can be used to predict the reversibility of the testicular lesion and other potential toxic effects of DBCP on human health.

The toxic effects of halogenated compounds on male germ cells and the biological consequences of such effects on subsequent generations, is of interest to the National Institute of Environmental Health Sciences - particularly with regard to 2,4,-dichlorophenoxyacetate (2,4-D), 2,4,5-trichlorophenoxyacetate (2,4,5-T) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), components of the herbicide Agent Orange. Thus, this project is of direct significance to the programs of the Institute.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30101-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Renal Function Tests as Indicators of Nephrotoxicity in Male, Fischer 344 rats.

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

PI:	William M. Kluwe	Staff Fellow	EBB NIEHS
OTHER:	Frank Harrington	Biological Laboratory Tech.	EBB NIEHS
	Ralph Wilson	Biological Laboratory Tech.	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Biochemical Toxicology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.50	.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)

Several tests of renal function are being conducted on rats treated once or repeatedly with a diverse group of chemical nephrotoxicants (biphenyl, carbon tetrachloride, mercuric chloride) to compare the relative sensitivities and versatilities of the various tests in detecting chemical nephropathy. In addition, quantitative urinalyses are being conducted serially on rats treated with known nephrotoxic chemicals to determine if refractoriness to kidney injury occurs during repeated (subacute, subchronic) dosing experiments.

The primary purpose of this project is to evaluate the usefulness of various renal function tests in subacute and subchronic toxicity studies and to compare alterations in function to changes in microscopic renal morphology. A secondary purpose is to develop improved testing methodologies.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to evaluate the general usefulness in rodent toxicity studies of various kidney function tests. Four criteria for such tests are sensitivity (ability to detect renal injury at the lowest effective dose of toxicant), versatility (ability to detect injury caused by several different types of nephrotoxicants), precision and ease of performance. Therefore, the tests are compared to each other and to histological analysis of the renal tissue to identify the most advantageous test procedures, based on their relative abilities to fulfill the four criteria listed.

METHODS EMPLOYED: In addition to standard urinalyses and serum analyses, the urinary excretions of protein (total), glucose, electrolytes and several enzymes are measured over a 16 hr collection period and expressed relative to body weight. Also being used are tests that more nearly measure the total functional capacity of the kidney, such as ability to concentrate urine during water deprivation and ability of renal tubular cells to actively (energy-dependent) accumulate organic ions in vitro.

MAJOR FINDINGS AND PROPOSED COURSE: The most advantageous test procedures, as judged by the criteria listed previously, following subacute nephrotoxicant exposure, appeared to be those which measured total renal capacity, such as urine concentrating ability during water deprivation and the accumulation of organic ions by renal tubular cells in vitro. Less advantageous, but still usefull, were histological examination of kidney morphology, gross kidney weight, 16 hr urine volume and specific gravity and the presence of blood (hemoglobin) in the urine (hematuria). Urinary enzyme excretions, standard urinalyses such as protein and glucose concentrations and standard serum analyses such as sodium, potassium, urea nitrogen and creatinine concentrations were of little value in detecting kidney injury.

Single dose studies revealed that most toxicant-induced alterations in urine composition are short-lived (up to 48 hr), but others can still be detected 8 days after toxicant exposure. The general utilities of both the transient and the longer-lived changes in urine composition in monitoring renal function are currently being evaluated in repeated-dose studies.

In addition to continuing evaluation and refinement (including automation) of the test procedures currently under study, the development of alternate methodologies is to be investigated. These will include the characterization and quantitative analyses of urinary proteins excreted both by normal and nephrotoxicant-treated animals, and the use of urine sediment analyses for diagnostic purposes in laboratory animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Renal function tests are important to basic toxicology both for confirming the presence (or absence) of kidney injury and for determining endpoints in mechanistic studies. The observation that nephrotoxicity is a common side

effect in the clinical use of drugs, but not in the preclinical toxicological evaluation of drugs (Peters, G., Proc. Eur. Soc. Drug Tox., 5:18, 1965), suggests that many of the widely-used methodologies for detecting kidney injury may be inadequate. Thus, this project is of significance to biomedical research and safety evaluation in general. In addition, the basic experimental designs in this project closely resemble those utilized by the National Toxicology Program (NTP) for subacute and subchronic toxicity studies and are directly relevant to the selection of testing methodologies for use by the NTP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30102-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Interactions Between Haloalkane 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

PI:	William M. Kluwe	Staff Fellow	EBB NIEHS
OTHER:	Frank Harrington	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Biochemical Toxicology

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)

Renal tissue (slices of renal cortex or separated renal tubular cells) is incubated with selected nephrotoxicants in sealed flasks containing a nutritional, physiological medium. Biochemical and physiological processes directly involved in the normal functioning of renal tubular cells are monitored to elucidate the effects of the toxicants on the cells. By altering the composition of the medium (e.g., pH, pCO_2, cofactor concentrations) and the physiological status of the cells (e.g., glutathione-depleted, microsomal enzyme-induced), the same system is used to study the biochemical/molecular mechanisms of chemical nephropathy and the metabolism of the toxicants by the kidney.

Correlative studies are conducted with intact animals to determine the roles of non-renal factors (e.g., toxicant distribution, non-renal toxicant metabolism, extrarenal organ damage) in the pathophysiology of the kidney injury.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are as follows:

1. Identify indices of tubular injury that can be produced totally *in vitro*.
2. Establish concentration-response relationships for nephrotoxicants and *in vitro* cell injury.
3. Determine the relationships between toxicant metabolism by renal cells and toxicant injury to renal cells *in vitro*.
4. Investigate the usefulness of the *in vitro* technique as a screen for potential nephrotoxicants.
5. Correlate *in vitro* cytotoxicity with *in vivo* kidney injury.

METHODS EMPLOYED: Renal cortical tissue is obtained from rats by removal of thin slices from the surface of the kidney or by enzymatic digestion of the interstitial tissue and isolation of the separated tubular cells. The tissue preparation is then incubated with selected nephrotoxic chemicals for varying periods of time in a balanced salt solution or a nutritional growth medium. A variety of different atmospheres (e.g., 95% O_2:5% CO_2, 100% O_2, 100% N_2) are used. Selected enzymatic activities, coordinated cellular functions (e.g., gluconeogenesis, oxygen utilization) and intracellular concentrations of various compounds (e.g., potassium, glutathione, ATP) are measured to assess cell viability. Toxicant metabolites are identified, and rates of biotransformation measured by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Several nephrotoxic haloalkane chemicals (e.g., 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, hexachloro-1,3-butadiene) were shown to cause concentration-dependent depressions, *in vitro*, of cellular functions such as gluconeogenesis, oxygen utilization, organic ion transport and maintenance of intracellular-to-extracellular inorganic ion gradients. A correlation between *in vitro* cytotoxic potency and nephrotoxic potential (or potency) in intact animals was also identified for haloalkane chemicals. These findings indicate that the *in vitro* technique may provide a valid system in which to study the mechanisms of chemical nephrotoxicity, and may also be useful as a screen for identifying compounds with nephrotoxic potential.

The relationships between renal biotransformation of nephrotoxic haloalkane chemicals and the development of tubular injury *in vitro* will be studied. Toxic and non-toxic products of renal haloalkane metabolism *in vitro* will be identified and compared with urinary metabolites from intact, haloalkane-treated animals. At some time in the future, results using renal tissue from several species of experimental animals will be compared with that using human renal tissue to assess the appropriateness of the various animal models. The probable source of human tissue would be viable kidneys considered unsuitable for transplantation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
There is growing interest in the use of *in vitro* techniques to screen for chemical toxicity and to study molecular mechanisms of tissue injury. Although most *in vitro* system currently utilize bacterial, tumor or mammalian liver cells, preparations of kidney cells are obviously preferable for the study of chemical nephropathy. The development of an *in vitro* system for studying the toxic effects of chemicals on renal tubular cells, therefore, is of interest to biomedical research, in general, and to investigative renal toxicology, in particular.

A great many environmental pollutants and industrial and agricultural chemicals exhibit nephrotoxic potential.. Thus, systems for studying the mechanisms of chemical nephropathies are of interes to the National Institute of Environmental Health Sciences and federal regulatory agencies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30103-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Six-Month Exposure of a 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	EBB NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	J.D. Allen	Biological Laboratory Technician	EBB NIEHS
	D.L. Myers	Biological Laboratory Technician	EBB NIEHS
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	0.9	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)

Subchronic and chronic studies with Firemaster FF-1 (mixture of polybrominated biphenyls) were conducted in both rats and mice of both sexes. The animals were given 125 oral doses of Firemaster FF-1 over a 6 month period at 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg/day (5 da/wk). The animals were sacrificed at the end of six-month treatment and the following parameters were studied: food consumption, body weight gain, hematology, clinical pathology, anatomic pathology and tissue porphyrins. Animals from each dose group are being held for life-time observations. The purpose of these toxicological studies is to determine the subchronic and chronic effects from exposure to Firemaster FF-1 in the rat and mouse.

PHS-6040
(Rev. 10-76)

METHODS EMPLOYED: Firemaster FF-1 (Lot No. 1312 FT) was given to rats and mice 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/day. Rats and mice were killed after 6 month treatment, organ were weighed and tissues were collected for histopathologic examination. Representative animals from the 6 month exposure were held for additional 23 months to observe the long term effect of Firemaster FF-1 exposure.

MAJOR FINDINGS AND PROPOSED COURSE: Although there was no significant difference in food consumption between treated and control animals, there was a dose related decrease in body weight gain in both male and female rats and male mice. Thymus weights were significantly decreased in all rats exposed to 0.3 mg/kg or more of FF-1. Dose related hepatotoxic effects were observed in both rats and mice which were characterized by marked increase in liver weight with accentuation of hepatic lobular markings. Microscopically, there were moderate to marked swelling, disorganization and single cell necrosis of hepatocytes, fatty infiltration, bile duct proliferation and presence of atypical foci in the liver. Excess porphyrin accumulation in the liver, teeth and bones of both treated rats and mice was denoted by reddish pink fluorescence under ultraviolet light. There was a significant dose related increase in the serum levels of gamma glutamyl transpeptidase and decrease in the serum glucose levels. The studies on rats and mice kept for an additional period after 6 month exposures are still in progress. However, preliminary examination of the data indicated a remarkably high incidence of hepatic neoplasm in exposed rats compared with the controls (Table 1).

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. (in press).

Gupta, B.N., and Moore, J.A.: Toxicological assessments of a commercial polybrominated biphenyl mixture (Firemaster FF-1) in the rat. Am. J. Vet. Res. 40: 1458-1468, 1979.

TABLE 1. Incidence of neoplastic lesions in the liver of Fischer 344/N rats exposed to Firemaster FF-1 for 6 months and observed for an additional 23 months

Dose mg/kg	Sex	No. examined	Rats positive for atypical foci/nodules			Hepato, cholangio or hepatocholangio carcinoma		
			No.	%	Spontaneous (%)*	No.	%	Spontaneous (%)*
0	M	33	1	3	1.3	0	0	0.44
0	F	20	2	10	2.7	0	0	0.39
0.1	M	40	3	8	-	2	5	-
0.1	F	21	2	10	-	0	0	-
0.3	M	40	12	30	-	1	3	-
0.3	F	21	1	5	-	0	0	-
1.0	M	32	16	50	-	1	3	-
1.0	F	11	2	18	-	1	9	-
3.0	M	33	14	42	-	7	21	-
3.0	F	19	8	42	-	3	16	-
10.0	M	31	15	48	-	7	23	-
10.0	F	20	7	35	-	12	60	-

* Goodman, et al.: Neoplastic and nonneoplastic lesion in aging F344 rat. Toxicol. Appl. Pharmacol. 48:237-248, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30104-01 EBB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Use of Whole-Body Sagittal Histologic Sections in Fetal Pathology of Pentachloroanisole

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	EBB NIEHS
OTHER:	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	T.A. Marks	Senior Toxicologist	RTI

COOPERATING UNITS (if any)

Chemistry and Life Sciences Branch, Research Triangle Institute, Research Triangle Park, North Carolina

LAB/BRANCH

Environmental Biology Branch

SECTION

Comparative Pathology 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)

To determine the fetal toxicity of pentachloroanisole (PCA), pregnant mice were treated orally on day 6 through 15 of gestation period. Fetuses were obtained by cesarian section on day 18 of gestation and examined histologically. The study is in progress.

PHS-6040
Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pentachloroanisole (PCA) was given orally to CD-1 pregnant mice on day 6 through 15 of gestation. The pregnant dams were sacrificed on day 18, fetuses were removed and fixed in buffered, neutral 10% formalin. After 3 to 4 hours, 0.1 to 0.2 ml of the formalin solution was injected intraperitoneally for better fixation of visceral organs. Several millimeters thick skin and subcutaneous tissues of each fetus were sliced off sagittally from both left and right sides. The trimmed fetuses were routinely embedded in paraffin for microscopic examination. Three subserial sagittal sections, one each from the left, middle and right side of the body, were cut at 6 μm thick and stained with hematoxylin and eosin.

MAJOR FINDINGS AND PROPOSED COURSE: Most vital organs such as lung, liver, kidney, heart and brain were present in the whole body sections.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ubiquitous distribution of pentachlorophenol in the environment requires the study of the toxic effects of this compound as well as its metabolites. Although a considerable amount of research has been conducted on pentachlorophenol, little is known about pentachloroanisole. The major metabolite (compound) formed during the chemical, microbiological and photochemical degradation process in the environment. Therefore, it is important to determine the effects of PCA on the developing fetus.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30105-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
OTHER:	J.H. Dean	Immunologist	EBB NIEHS
	M.I. Luster	Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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), 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 polybrominated biphenyl (PBB), tetrachlorodibenzo-p-dioxin (TCDD), diethylstilbestrol (DES), indomethacin and Fryol FR-2. The chemical presently under evaluation is benzpyrene. New chemicals will be evaluated as part of the overall immunotoxicology program.

PHS-6040
(Rev. 10-76)

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. 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.

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. Project is continuing.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30106-01 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

PI:	J.H. Dean	Immunobiology Program Leader	NIEHS EBB
	M.I. Luster	Research Microbiologist	NIEHS LEC
	G.A. Boorman	Veterinary Pathologist	NIEHS EBB
OTHER:	L.D. Lauer	Biological Laboratory Technician	NIEHS EBB
	L.D. Lawson	Biological Laboratory Technician	NIEHS EBB
	R.E. Wilson	Biological Laboratory Technician	NIEHS EBB

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Immunobiology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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 function 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. These studies will hopefully allow correlations between changes in immunological parameters to provide prognosticators of alterations in host resistance to bacterial and viral diseases or tumors.

PROJECT DESCRIPTION

METHODS EMPLOYED: The assays that are being utilized or under development to assess immunological dysfunction or altered host resistance following chemical exposure are listed in the testing battery described in Table 1. Major emphasis has been placed on assays that can be automated, routinized and require only microquantities of cells or blood.

MAJOR FINDINGS AND PROPOSED COURSE: Studies with the PYB6 tumor susceptibility and *Listeria monocytogenes* infectivity assays have demonstrated that $B_6C_3F_1$ mice with normal unimpaired host resistance and normal T-lymphocyte parameters can resist a rather large challenge dose of syngeneic tumor cells ($5\text{-}10\times10^3$) or *Listeria* (1×10^6) with a mortality frequency of only 10% (i.e., TD_{10} or LD_{10}). The mechanism by which the unimmunized host resists this challenge dose of syngeneic tumor cells or bacteria are known to involve immune mechanisms requiring immunocompetent T-cells and macrophages. Following exposure of mice to the known immunosuppressive agent cyclophosphamide or chemicals of environmental concern including diethylstilbestrol (DES) or TCDD, host resistance was severely impaired resulting in a high frequency of PYB6 tumors or *Listeria* mortality (80-100%). The immune alterations induced by these chemicals also significantly affected the number of lung tumor cells which grew into tumor foci in the lungs following an iv challenge with Lewis lung tumor cells, as measured by incorporation of ^{125}IUDR. The isotopic procedure using lung tumor cell lines is currently under development and evaluation as a more rapid and quantitated means of assessing altered host resistance. Likewise, other bacteria, viruses and parasites will be evaluated as model systems to study chemical induced immunotoxicity.

Procedures to assess T-lymphocyte, B-lymphocyte, bone marrow progenitor cell and macrophage (MØ) function have been established and refined in our laboratories. During the past year the immunology assay battery has been applied to study immune dysfunction following exposure of $B_6C_3F_1$ mice to cyclophosphamide (CY), DES, TCDD, orthophenylphenol, Fyrol 2B and benzepyrene (BP). Immune dysfunction was observed following chemical exposure to CY, DES, TCDD and BP which involved dysfunction of various cell types including T-cells, MØ's and bone marrow progenitor cells with antibody producing cells (B-cells) being less frequently effected. Alterations in the *in vitro* assays of T-cell and MØ function also appear to correlate with alterations in host resistance described above. Emphasis is currently being placed on developing assays for natural killer cells, erythrocyte and megakaryocyte progenitor cells and other MØ functions. Chemicals under evaluation at present include known carcinogens such as benzepyrene and DES to address the concern that some environmental carcinogens operate through immunological mechanisms.

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, orthophenylphenol, 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

Dean, J.H., Padarathsingh, M.L., and Jerrells, T.R.: Application of immunocompetence assays for defining immunosuppression. Ann. NY Acad. Sci. 320: 579-590, 1979.

Luster, M.I.: Assessment of immunological alterations caused by halogenated aromatic hydrocarbons. Ann. NY Acad. Sci. 320: 572-578, 1979.

Dean, J.H., Padarathsingh, M.L., and Jerrells, T.R.: Assessment of immunobiological effects induced by chemicals, drugs or food additives. I. Tier testing and screening approach. Drug and Chem. Tox. 2: 5-17, 1979.

Dean, J.H., Padarathsingh, M.L., Jerrells, T.R., Keys, L., and Northing, J.W.: Assessment of immunobiological effects induced by chemicals, drugs or food additives. II. Studies with cyclophosphamide. Drug and Chem. Tox. 2: 133-153, 1979.

Luster, M.I., Faith, R.E., McLachlan, J.A., and Clark, G.: Effect of in utero exposure to diethylstilbestrol on the immune response in mice. Toxicol. Appl. Pharmacol. 47: 279-285, 1979.

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).

Dean, J.H., Luster, M.I., Boorman, G.A., and Padarathsingh, M.L.: Host resistance models as an endpoint for assessing immune alterations following chemical exposure. Environ. Protect. Agency J. 400 Series (in press).

Luster, M.I., Boorman, G.A., Dean, J.H., Lawson, L.D., Wilson, R.E., and Haseman, J.K.: Immunological alterations in mice following acute adult exposure to diethylstilbestrol. In Dean, J.H., and Padarathsingh, M.L. (Eds.): Biological Relevance of Immunosuppression. New York, Van Nostrand Reinhold (in press).

Dean, J.H., Luster, M.I., Boorman, G.A., Padarathsingh, M.L., Luebke, R.E., and Clements, M.E.: Host resistance models as endpoints in assessing immune alterations following chemical exposure: studies with diethylstilbestrol, cyclophosphamide and 2,3,7,8-tetrachlorodibenzo-p-dioxin. In Dean, J.H., and Padarathsingh, M.L. (Eds.): Biological Relevance of Immunosuppression. New York, Van Nostrand Reinhold (in press).

TABLE 1
PARAMETERS TO EXAMINE TO MEASURE IMMUNOLOGIC DYSFUNCTION FOLLOWING CHEMICAL EXPOSURE IN RODENTS[1]

Parameter	Procedure Performed
Pathotoxicology	Hematology Profile-hemoglobin, RBC, WBC, differential Liver Chemistries-SGPT, triglycerides, cholesterol Serum Proteins-albumin, globulin, A/G, total proteins Lymphoid Organ Weights-spleen and thymus Histology-liver, thymus, lung, kidney, spleen
Host Resistance	Tumor Challenge-TD_{10-20} *Listeria monocytogenes* Challenge-LD_{10-20} Endotoxin hypersensitivity
Delayed Hypersensitivity	Radiometric DHR to T-cell dependent antigens
Lymphocyte Proliferation	One-way mixed leukocyte cultures Mitogens-PHA, Con A, LPS
Humoral Immunity	Immunoglobulin levels Titer of serum antibody to T-dependent antigen Plaque forming cell response-T-dependent antigen
Macrophage Function	Phagocytic index Lysosomal enzymes Cytostasis of tumor target cells Cytolysis of tumor target cells
Bone Marrow Colony Forming Units	CFU-S-multipotent stem cells CFU-GM-granulocyte/macrophage progenitor CFU-M-megakarocytes progenitor CFU-E-erythrocytes progenitor Histology, myeloid/erythroid ratio and bone marrow cell count

1 The assays described in this panel can all be performed on six groups of animals containing 20-40 animals/group.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35004-02 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	EBB NIEHS
	Amin Nomeir	Visiting Fellow	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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)

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. 10-76)

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 synthetized 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) Each of these compounds is readily absorbed from both the skin and intestines and distribution is similar regardless of the route of exposure.

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.

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.

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. In press.

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. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-07 EBB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	EBB NIEHS
Other:	Daniel B. Tuey	Staff Fellow	BB NIEHS
	Linda S. Birnbaum	Staff Fellow	EBB NIEHS
	Gary M. Decad	Staff Fellow	EBB NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS; Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH

Environmental Biology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.5	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 ke·

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 for simple halogenated aromatics the rate of metabolism is limited by the availability of adjacent unsubstituted carbon atoms which are thought to facilitate metabolism via arene oxide intermediates. This work has also established that halogenated aromatics are readily absorbed from the gastrointestinal tract, that those compounds which are not 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. This work has demonstrated that acute as well as chronic toxicity may be related to the exposed animals ability to metabolize and excrete the toxic compound, and that for tetrachlorodibenzofuran (TCDF) chronic toxicity may not be manifest prior to the accumulation of a critical body burden.

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) 2,3,7,8-Tetrachlorodibenzodioxin (TCDF) is readily absorbed from the gastrointestinal tracts of rats and guinea pigs. Absorption is at least 90% complete and is unaffected by the size of the dose in the range studied. Following either oral or iv administration TCDF is initially concentrated in the liver of rats, guinea pigs and monkeys. The rat metabolizes TCDF at an appreciable rate and excretes the metabolites in the bile and ultimately in the feces. The guinea pig apparently unable to metabolize TCDF, stores it for an extended period of time and is very sensitive to intoxication by TCDF. The monkey metabolizes and excretes TCDF at a rate which is intermediate between that of the rat and guinea pig, however, it has been possible to isolate a metabolite from monkey feces. With multiple doses the guinea pig gains weight normally until a critical body burden is accumulated at which time the body weight declines rapidly and the symptoms of acute intoxication are observed.

2) 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 five fold 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.

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 rate at which they are metabolized, 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 knowledge by determining how other types of substitutions, in addition to halogens, effect the metabolism and disposition of organic compounds. In addition, the effort to elucidate the biochemical mechanism(s) by which halogenated hydrocarbons exert their action will be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated xenobiotics are the most toxic and persistent of the chemicals contaminating our environment. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and numerous industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man and may lead to more specific modes of treatment including better ways to accelerate removal of such compounds from the body.

PUBLICATIONS

Matthews, H. B. and Kato, S.: The metabolism and disposition of halogenated aromatics. Ann. N. Y. Acad. Sci. 320:131-137, 1979.

Morales, N. M., Tuey, D. B., Colburn, W. A. and Matthews, H. B.: Pharmacokinetics of multiple oral doses of selected polychlorinated biphenyls in mice. Toxicol. Appl. Pharmacol. 48:397-407, 1979.

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Morales, N. M. and Matthews, H. B.: The role of metabolic activation in the binding of polychlorinated biphenyls to macromolecules, in vivo. Chem. Biol. Interact. 27:99-110, 1979.

Kato, S., McKinney, J. D. and Matthews, H. B.: Metabolism of symmetrical hexachlorobiphenyl isomers in the rat. Toxicol. Appl. Pharmacol. In press.

Matthews, H. B. and Tuey, D. B.: The effect of chlorine position on the distribution and excretion of four hexachlorobiphenyl isomers. Toxicol. Appl. Pharmacol. In press.

Abdel-Hamid, F. M., Moore, J. A. and Matthews, H. B.: A comparative study of 3,4,3',4'-tetrachlorobiphenyl in male and female rats and female monkeys. J. Toxicol. Environ. Health. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80020-08 EBB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents

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

PI:	H.F. Lam	Visiting Fellow	EBB NIEHS
	J. Takezawa	Visiting Fellow	EBB NIEHS
OTHER:	E.W. Van Stee	Physiologist	EBB NIEHS
	R.A. Sloane	Bio Lab Technician	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	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)

Changes in pulmonary function resulting from inhalation of airborne pollutants are being estimated using a whole body pressure plethysmograph constructed for use with small animals. Both acute and chronic exposure to chemicals, singly and in combination with other compounds result in functional changes. Changes in pulmonary mechanics (e.g. resistance, compliance), spirometry (e.g. lung volumes, frequency of breathing), heart rate, and pulmonary morphology will be estimated.

PHS-6040
(Rev. 10-76)

METHODS EMPLOYED: We have established the methods to assess the changes in pulmonary function induced by environmental toxicants. Our methods include single breath diffusing capacity for carbon monoxide in the lung (D_{LCO}), neon dilution to measure lung volumes such as residual volume (RV), functional residual capacity (FRC), and total lung capacity (TLC), and methods to measure static compliance and resistance in small laboratory animals.

MAJOR FINDINGS AND PROPOSED COURSE: Paraquat intoxication in its initial stage is characterized histologically in the lungs by atelectasis, hyaline membrane formation, alveolar edema and vascular hemorrhage often into the interstitium or air spaces. Information on the functional modification of paraquat-damaged lungs has been lacking.

We evaluated lung volumes, single breath diffusing capacity of the lungs for carbon monoxide (D_{LCO}) and static lung compliance ($D_{st}(L)$) in rats treated with paraquat or diquat. Measurements were made 12, 24, 48, and 72h after treatment. Paraquat by intratracheal instillation (IT) 0.5 mg/kg or by intraperitoneal injection (IP) 27 mg/kg significantly decreased ($P<0.01$) the body weight (absolute wet lung weight), total lung capacity (TLC), vital capacity (VC), D_{LCO}, apparent alveolar volume (VA) and $C_{st}(L)$. Paraquat IP 27 mg/kg induced a transient increase followed by a decrease in functional residual capacity (FRC). At a lower dose level (13.5 mg) the effects of paraquat peaked at about 24h following treatment, causing a significantly decreased ($P<0.01$) in VC, TLC, VA, and $C_{st}(L)$. Diquat IP or IT had little effect on the lungs, However, diquat IP decreased body weight and caused a temporary, but significant decrease ($P<0.01$) in VC and $C_{st}(L)$, 24h after treatment. The data obtained are consistent with the known pathological changes seen in paraquat-damaged lungs in that, by both routes, paraquat caused severe lung damage associated with decreased elasticity of the lungs and thorax, destruction of gas exchanging alveolar surfaces, and edema. These changes were detected reliably by lung function measurements.

We evaluated lung volumes, single breath diffusing capacity of the lungs for carbon monoxide (D_{LCO}), static lung compliance ($D_{ST(L)}$), and total pulmonary resistance (R_L), in rats during treatment with various anesthetics. The treatments were as follows: sodium pentobarbital (PB) (IP, 50mg/kg); PB and atropine sulfate (ATR) (IP, 50 mg/kg; IM, 80 µg/kg); ketamine (K) (IM, 100 mg/kg); K and xylazene (IM, 100 mg/kg; IM, 25 mg/kg); ketaset plus (IM, 100 mg/kg); K and acetylpromazine maleate (IM, 100 mg/kg; IM 1 mg/kg); methoxyflurane (0.5-1.5 vol%); halothane (H) (1-2 vol%); H and ATR (1-2 vol%; IM 80 µg/kg). When compared with Group 1, minute ventilation was elevated in all other Groups, mainly the result of increased respiratory frequency. Vital capacity, total lung capacity, residual volume, and functional residual capacity were significantly increased ($P<.01$) in Groups 5 and 6. A slight but significant increase ($P<.05$) in D_{LCO} was present in Group 3 which could have been related to the Group's overall increased minute volume. $C_{ST(L)}$ was increased in Groups 3 and 8, and R_L in Groups 3, 6, and 8. The data obtained show that different anesthetics do not induce similar changes in the lung,

and that conflicting lung function values present in the literature could be the result, in part, of different anesthetics used.

The effects of the inhalation of morpholine on lung function in rats were studied. Animals were exposed to 2000 ppm of morpholine, 4 hours per day, for 4 days (acute study), or 450 ppm, 6 hours per day, 5 days a week, for 30 days (subacute study). Body weight (BW), lung weight (LW), residual volume (RV), vital capacity (VC), total lung capacity (TLC), and single breath diffusing capacity of the lung for carbon monoxide (D_{LCO}) were measured daily in the acute study and on the 30th day in the subacute study. Terminal body weights of exposed animals were lower than the controls. RV and TLC were increased, and D_{LCO} was decreased by both the acute and subacute exposures.

Histopathological examination revealed no distinctive morphologic changes that could account for the above changes directly. These data suggest that exposure to morpholine may cause an obstructive lung defect associated with small airway disease, and that these changes can only be detected distinctively by physiological measurements.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
This project has been terminated by the Intramural Research Program.

PUBLICATIONS

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 (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, 1980 all hamsters have died and the histopathology has been completed. The pathology findings are being reviewed by an independent pathologist and this review should be completed by July 1, 1980. The findings and final report should be completed by September 1, 1980.

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 21180
NIEHS-NO1-ES-5-2158

TITLE: Long Term Study on the Biological Effects of Ingested Asbestos in Rats

CONTRACTOR'S PROJECT DIRECTOR: Henry A. Rutter, Ph.D.

PROJECT OFFICER (NIEHS): J.A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $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 (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, 1980 most of the animal groups have been on test about 18 months. Therefore, little data is available at this time. This lifetime study is scheduled for another 6 months followed by pathology evaluation and data analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas of the United States are contaminated with asbestos fibers as are several common food items. Because of this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

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) Benzidine and six benzidine based dyes have been studied in the dog and the rat. In each of these studies dogs excreted free benzidine in the urine following oral administration of benzidine based dyes. The % of the dye dose excreted as benzidine was not large, but it was comparable to the benzidine excreted in urine following oral administration of a similar dose of pure benzidine. Rats excreted traces of benzidine in urine following the administration of benzidine, but did not excrete benzidine in the urine following administration of the benzidine based dyes.

2) Two 3,3'-dimethoxybenzidine based dyes have been studied in dogs and rats. Both dogs and rats excreted free dimethoxybenzidine in the urine following oral administration of these dyes. The amount of 3,3'-dimethoxybenzidine excreted was less than that excreted following the administration of pure 3,3'-dimethoxybenzidine, but greater than the amount of free 3,3'-dimethoxybenzidine administered with the dye.

3) Four 3,3'-dimethylbenzidine based dyes have been studied in dogs and rats. Both dogs and rats excreted 3,3'-dimethylbenzidine in urine following the oral administration of two of these dyes, Direct Blue 25 and Acid Red 114, but did not excrete 3,3'-dimethylbenzidine in the urine following the administration of two others, Direct Red 2 and Direct Red 39.

4) The studies described above were facilitated by the development of improved analytical techniques for the detection and quantitation of these compounds in urine.

PROPOSED COURSE: 1) The major finding described above were obtained without the use of a radiolabel and therefore were limited to straightforward detection and reporting. Current plans call for the use of radiolabeled compounds to accurately quantitate gastrointestinal absorption, distribution metabolism and excretion of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine. Radiolabeled benzidine will also be used to synthesize a radiolabeled benzidine based dye to be used in similar studies of absorption, distribution, metabolism and excretion. The 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.

2) Additional compounds or classes of compounds will be studied in support of the NTP and intramural research of the NIEHS.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies of such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data which will complement studies of toxic xenobiotics under the NTP or in the NIEHS intramural program.

PUBLICATIONS

Lynn, R. K., Wong, K., 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. In press.

Arizona Board of Regents
University of Arizona
Tucson, Arizona 85724
NO1-ES-8-2130

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

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 similarly, sacrificed in a serial manner and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest and metabolites. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) The metabolism of three polychlorinated biphenyls (PCBs) (4,4'-dichloro-; 2,3,6,2',3',6'-hexachloro-; and 2,4,5,2',4',5'-hexachlorobiphenyl) have been studied in vitro and in vivo in three species--the rat, dog and monkey. The PCBs were chosen to represent compounds which range from relatively unchlorinated to highly chlorinated compounds which are readily metabolized as well as a compound which is very resistant to metabolism. The results of these studies indicate that it may be possible to predict in vivo metabolism and disposition of foreign compounds based on rates of in vitro metabolism.

2) The metabolism and disposition of chlorpheniramine maleate has been studied in the rat. The results of these studies indicate that chlorpheniramine maleate is readily absorbed from the gastrointestinal tract, rapidly metabolized and excreted, and poses little threat of bioaccumulation when administered at therapeutic doses.

PROPOSED COURSE: 1) The work on *in vitro* vs *in vivo* metabolism will be continued for sometime in an effort to refine the technique and hopefully provide data which will permit the extrapolation of these results to man.

2) Metabolism and disposition studies of acrylamide, p-chloroanaline and p-nitrotoluene are scheduled to be done in support of work being done on these compounds at the NIEHS and by NTP contractors.

3) 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.

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., Pharmacologist, Environmental Biology Branch

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $443,728.00

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 end points.

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 offsprings 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 toxico-pathologic 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 end points 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 week 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 recently, Kepone has been withdrawn from this study. The following is the status of study on the individual chemicals:

Phenytoin: After completion of acute and repeated dose studies the 90 day sub-chronic study in rats and mice were initiated and recently completed. All dosed rats survived although clinical abnormalities were noted in those exposed to the highest three dose levels. These abnormalities included porphynin pigmentation of eyes and nose, coat discoloration, and piloerection. All rat groups showed a net weight gain over the study period although the weight gain of the male and female rats in the highest dose group was only about one half that of controls.

In mice, all males exposed to the highest dose level (1200 ppm) died or were terminated in a moribund condition, only the females at that dose survived to necropsy. In both sexes, convulsions, hunched posture and unstable gait were noted at this dosage. All groups exposed to 600 ppm or less gained weight over the period although a clear dose-related depression in body weight gain was seen in both sexes. Females appeared to tolerate higher doses than males in that body weight gain was closer to control levels at 300 and 600 ppm in that sex. Among rats, no gross abnormalities were apparent at necropsy. Among mice, changes observed at necropsy included abdominal distension, adrenal gland enlargement, bladder distension and corneal opacity. These changes were most prevalent in mice given 1200 ppm. More definitive interpretations will be possible when histologic studies now underway are complete.

Ethylenethiourea (ETU): The acute and repeated dose studies have been completed. Under 90 day sub-chronic study, male and female Fischer 344 rats and B6C3F1 mice are presently dosed in feed with this chemical. Rats are exposed to 750, 500, 250, 125 and 60 ppm and mice to 2000, 1000, 500, 250 and 125 ppm along with control groups of both species receiving normal feed. The sub-chronic study is scheduled for termination in the next 30 days.

Firemaster FF-1: The studies with Firemaster FF-1, a PBB mixture, have been postponed pending approval of a revised protocol for these studies and contract modifications for revision of the cost estimate. All contract modification documents have been completed and final approval from the contract laboratory is awaited. The studies with this chemical will begin with MND determination since pre-chronic work has already been completed at NIEHS. The laboratory studies will begin after the facilities modifications of contract labs. are completed for additional safety and health precautions needed for this portion of the project.

It is anticipated that after the review of pre-chronic data on Phenytoin and Ethylenethiourea, the MND determinations for these chemicals will begin. The chronic (2 year) studies will be initiated after the determinations of MND for the chemicals is completed.

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.

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 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 immune toxicity.

In summary, 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) If immune dysfunction is found, to investigate whether other reported symptoms, signs or conditions occur more frequently in those persons with such dysfunction than without; and, 4) To characterize the nature of the immune dysfunction.

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: The Michigan and Wisconsin populations to be studied have been contacted and logistics have been developed for the pending visits and evaluations of these individuals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The relationship between PBB exposure, current PBB body burden and altered immune function in man remains undefined. The health implications of this observed T-lymphocyte dysfunction may have serious long-term consequences on immune surveillance against infectious agents and neoplastically transformed cells if confirmed.

PUBLICATIONS

Roboz, J.P., Suzuki, R., Bekesi, J.G., Holland, J.F., Roseman, K., and Selikoff, I.: Mass spectrometric identification and quantification of PBB in blood compartments of exposed Michigan Chemical Workers. J. Env. Path. Tox. 3:363, 1980.

Bekesi, J.G., Anderson, H., Roboz, J.P., and Selikoff, I.J.: Investigation of the immunobiological effects of PBB in Michigan farmers. In Dean, J.H., and Padarathsingh, M.L. (Eds.): Biological Relevance of Immunocompetence. New York, Van Norstrand Reinhold (in press).

NORTHROP SERVICES, INC. - Research Triangle Park, NC 27709
NIEHS NO1-ES-79-0009

TITLE: Animal Research on the Inhalation Toxicology of Environmental Chemicals

CONTRACTOR'S PROJECT DIRECTOR: Bernard Adkins, Ph.D.

PROJECT OFFICER (NIEHS): E.W. Van Stee, D.V.M., Ph.D.

DATE CONTRACT INITIATED: June 29, 1979

CURRENT LEVEL (5 years): $2,663,653.00

PROJECT DESCRIPTION

OBJECTIVES: Conduct research in the inhalation toxicology of environmental chemicals using dynamic flow-through inhalation chambers designed for use with small laboratory animals. Exposures are conducted intermittently since the inhalation facility is not equipped for 24-hour inhalation exposures. Generate, monitor, characterize and control the generation of solid aerosols of asbestos and related natural and man-made fibers in 1-4 inhalation chambers as specified to support the research program of the 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 tudy in which groups of rats and hamsters receive NO_2 by inhalation and ,6-dimethylmorpholine (DMM) in the drinking water, air plus DMM, NO_2 and plain drinking water, or air plus plain drinking water. Conduct an inhalation study imed at testing the biological inertness of SF_6 for the purpose of evaluating the possible use of SF_6 as tracer in inhalation experiments.

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 into early 1981. Computer-assisted operation of the facility should e well-along by the end of 1981. A detailed protocol for the NO_2-DMM study been written and reviewed. Definitive animal exposures are projected to gin during mid-1980. A preliminary palatability study has been completed in which groups of animals were given deionized water (controls), 0.01%, 0.05%, 0.10%, 0.50%, or 1.00% 2,6-dimethylmopholine in the drinking water for up to 33 days. A sharp divergence in responses occurred between the groups treated with 0.1% and 0.5%, respectively, Tissues have yet to be examined for histopathological confirmation, but based on water consumption and animal growth charts, the decision has been tentatively made to begin the definitive study with 0.1% DMM, the highest concentration that did not seriously impair food and water consumption and weight gains. Animals fared better on DMM in water as compared with DMM in water neutralized to pH 6.5-7.0 with HCl. DMM-water treatments will be continuous. Animals treated with NO_2 will be exposed to approximately 10 ppm, 6 hr/da, 5 da/wk, 52 wk/yr, for 2 yr or until death. Tissues will be examined for gross and microscopic evidence of tumorigenesis related to the treatments. If the

results of this study support the idea of a carcinogenic cascade initiated by the in vivo interaction of NO_2 and DMM to N-nitroso-2,6-dimethylmorpholine, another study will be proposed in which diets are enhanced with antioxidants like ascorbic acid which may interfere with the in vivo synthesis of nitrosamines.

A research protocol has been prepared describing a study of the indirect monitoring of compounds of interest in inhalation exposure chambers by following the concentration of a tracer gas (SF_6) present in fixed ratio with the compound of interest. This concept could be employed for studies in which hard-to-monitor substances like asbestos or oxides of nitrogen were under study. For example, the detection of SF_6 in a chamber room in which the chamber gas had been spiked with SF_6 might allow the practically instantaneous detection of potentially highly hazardous system leaks that could endanger personnel in the vicinity. Reliance on detection of the fibers themselves can lead to dangerous delays in leak detection. Also, if SF_6 were shown not to interfere with the outcome of an experiment, it could conceivably be employed to monitor continuously chamber NO_2 when introduced into the chamber at a fixed ratio with the NO_2 thus obviating the need for the use of intermittent sampling devices (chemiluminescent) in a computer-assisted operation of the chambers. Rats, mice and hamsters were exposed 6 hr/da, 5 da/wk, for 3 weeks to air (controls), 22-24 ppm NO_2, 22-24 ppm SF_6, or 22-24 ppm NO_2 plus 22-24 ppm SF_6. Animals were weighed weekly, and at the end of the experiment, the lungs were excised and fixed by inflation for routine histopathological examination, which results are not yet available. Analysis of weight data indicates a possibility that the rat weight gains may have been retarded in the combination treatment group. This idea will be pursued further. Groups of rats and hamsters will be exposed for at least 12 weeks, to 10, 20 or 40 ppm of SF_6 for 6 hr/da, 5 da/wk. Animals will be weighed weekly and lung histopathology performed by routine methods at the conclusion.

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 inhalation facility operation as well as the documentation of that operation, and greatly reduces manpower requirements, thus reducing 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 given morpholine by gavage and exposed to NO_2 by inhalation has been reported. The biological significance of the phenomenon has yet to be demonstrated. These studies form a complement to closely related studies already underway at NIEHS. If these studies yield a positive result they will represent documentation of a specific chemical link between exposure to NO_2 and tumor formation. Such studies further pave the way for efforts to be made to find ways of interfering with some element of the in vivo process, for example, through the augmentation of dietary antioxidants like ascorbic

acid. Studies with the inert tracer gas SF_6 may have a wide variety of applications in the future of inhalation technology, including improved safety through more rapid leak detection.

LABORATORY OF ANIMAL GENETICS

LABORATORY OF ANIMAL GENETICS
Summary Report

During the first year of its existence, the Laboratory of Animal Genetics has undergone some major changes. A research group involved in the study of gene structure and organization has been created while the group in Genetic Enzymology has been phased out with the transfer of the group leader to the Environmental Chemistry Branch. The program in Mammalian Genetics is being reevaluated, Population Genetics has taken a dramatic shift toward developing molecular techniques for determining genetic variability in populations, and the direction and focus of the work in Chemical Genetics has turned toward analysis of nucleic acid sequences as well as the proteins they encode. These changes are designed to unify the research efforts of the Laboratory in approaching the central questions concerning the nature of genes and what genes represent as targets for environmental mutagens.

The emphasis of the Laboratory's research program is on understanding fundamental mechanisms of how eukaryotic genes function and how they are regulated. Such knowledge is pivotal to the solution of problems of mutagenesis and carcinogenesis, birth defects and disease in general. If we are to understand the impact of environmental pollutants on living systems we must look at the molecular structure of genes and the mechanisms involved in gene action. We can then examine the effects of mutagenic changes on the development and function of cells and individuals and the impact such changes have on the genetic architecture of populations.

Gene Structure

A major effort in this group centers on the study of the structure and the mechanism of regulation of the white locus in Drosophila. This locus is an excellent model system for answering questions about gene organization and regulation because of the great amount of genetic information that has been collected about it and the large number of mutant alleles and chromosomal aberrations that exist at the locus. We have determined that regulation of the locus is accomplished through cooperative action of elements in the proximal part of the locus itself and the z locus located in the same chromosome. By recombinant DNA techniques, the white locus has been cloned and efforts are now being made to discover how the repression and activation of the locus is accomplished. The molecular structure of the gene is being studied and normal alleles are being compared to the structures of alleles that are not properly regulated.

This gene is also of great interest because several examples of highly mutable forms of the locus are known. Implicated in this state of greatly increased mutational activity are interspersed elements of DNA that are transposable from one site to another in a chromosome set. A major fraction of the mutant events appear to be deletions of the locus or other forms of chromosomal aberrations with a break at the site of integration of the transposable element. The cloning of the white locus now allows the study of this type of mutational event and a detailed examination of the structures of the transposable elements.

We can now attempt to understand at the nucleotide level the nature of the inserted sequence and how the mutational events occur. This information will be of tremendous importance to questions about mutagens and their actions on genes. Transposable elements could in fact be important factors in spontaneous mutation processes in eukaryotic chromosomes. Such elements are almost certainly present in higher eukaryotes, including humans, but at the present time we have little insight to how they function or how the respond to mutagens. This study is aimed at fulfilling an important part of the mission of the Laboratory by defining one of the fundamental properties of the eukaryotic gene as a mutable element.

The cloned segment of the white locus is also being used to study the molecular organization of the section of the X chromosome, 3A-3C that we have characterized so extensively in genetic and cytological terms. We want to know how many genes exist in this segment, how big they are, how they are arranged relative to the chromomeric pattern of the polytene chromosome and how they function in the development of the organism. We have a lot of information about the relative mutability of genes in this region and we want to see how those data correlate with molecular sizes and organizational patterns of these genes.

The second major effort in the gene structure group is focused on a genetic and biochemical characterization of the genes encoding subunits of the RNA polymerase II of Drosophila. The enzyme is a heteromultimer composed of approximately 10 subunits. Mutants of a locus that encodes one of the subunits confer resistance to α-amanitin. Using temperature sensitive alleles of this locus, several different approaches to identifying loci encoding other subunits are now underway. Because RNA polymerase II has a dominant role in one of the steps where gene regulation occurs, transcription, its characterization and how it interacts with the DNA being transcribed is of great practical and theoretical importance to understanding genetic control mechanisms.

Population Genetics

Objectives of the Population Genetics group are to gather base-line data about the nature and extent of genetic variability in natural populations and to use this information to model the effects of mutagens on the genetic structure of populations. The approach that the group has taken toward these goals is to develop techniques for determining genetic variability at the most fundamental level, the DNA molecule.

One of the projects utilizing electron heteroduplex analysis and restriction endonuclease digest mapping of mitochondrial DNAs of three Drosophila species has shown that there is significant interspecific divergence of these molecules and has allowed estimates of the levels of intraspecific variation in the mitochondrial DNAs.

A project completed during the year dealt with determination of the frequency of null alleles at loci encoding selected enzymes in Drosophila and the use of these data to examine how such alleles are maintained in populations.

Also completed was a project that examined mutants at several enzyme encoding loci in individuals exposed to chronic low-dose γ-irradiation. Mutants in-

duced were mainly point mutations in contrast to those associated with chromosomal aberrations. From the same irradiation series, induced recessive lethal rates were measured. This study indicated that chronic exposure to X rays produces a similar number of mutants per rad as acute exposure.

Mammalian Genetics

Because of limited resources and positions available to the Laboratory, the program in Mammalian Genetics has been cut back sharply to allow reevaluation of the projects and to determine how best to proceed toward the goals of understanding the nature of mammalian genes, how they function, how they are controlled and how they respond to mutagens. In keeping with the philosophy that the knowledge we seek must extend to the direct analysis of nucleic acids, we are trying to develop a strong group working on the molecular organization of mammalian genes. It is absolutely necessary, however, that the program include genetic characterization of mutants and developmental studies of their impacts on the whole organism.

Projects that have been phased out during the interim period were primarily concerned with the detection of recessive lethal mutations induced by chemicals. Mutants recovered from these studies will be maintained and studied to determine the time and mode of their action in development. The nature of the mutations, whether they are associated with chromosomal aberrations is also an important aspect of this program and involves the analysis of chromosomes from cultured cell lines of mutant-bearing individuals.

Cytogenetic studies involving sister chromatid exchange induction by selected mutagens have also been carried out under the mammalian genetics program. The development of a sensitive *in vivo* system for detection of genetic damage by drugs sensitive to host activation or detoxification is continuing through a collaborative effort with EPA.

Genetic Enzymology

The transfer of the Group Leader of Genetic Enzymology to the Environmental Chemistry Branch reflects the shifting emphasis of the Laboratory of Animal Genetics toward direct analysis of DNA sequences, supported by strong efforts in genetics, cytology and developmental genetics.

The work carried out in Genetic Enzymology involved the biochemical characterization of mutant gene products of Drosophila and Mus in order to understand the nature of naturally occurring and induced gene changes. It is anticipated that interaction between members of the Laboratory of Animal Genetics and the Environmental Chemistry Branch will allow continuation of the biochemical analyses of selected gene products.

Chemical Genetics

The major focus of this group has been on the amino acid sequencing of selected gene products in order to correlate functional properties of these proteins with their primary structure. Specifically the gene 32 DNA-binding protein of bacteriophage T4 has been about 85% sequenced and the complete structures

of major coat proteins of If1 and Ike have been worked out. The mammalian lactate dehydrogenase (LDH) isozymes of mouse muscle, heart and testis and rat testis have been fragmented and much of the sequencing done. Recently the group has extended the analysis of the LDH system to the DNA level. A system has been devised to clone the LDH gene by recombinant DNA techniques. The comparative structures of the isozymes and the ways they are expressed selectively in different tissues are important questions being investigated. The answers will be very useful in understanding how mammalian genes are organized, how they function and the nature of mutations that modify their function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60057-04 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Molecular Analyses of Mitochrondrial DNAs of Different Species of Drosophila

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

COOPERATING UNITS (if any)

D. Shah, Department of Microbiology, Cummings Life Science Center, Chicago, Illinois

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We have done preliminary characterization of mitochondrial DNAs isolated from three species of Drosophila. Electron microscope heteroduplex and restriction endonuclease analyses have revealed that these mtDNAs have undergone a significant evolutionary divergence in their nucleotide sequences. We are constructing specific cleavage maps of these mtDNAs using various restriction endonucleases. Restriction endonucleases recognize specific base sequences within a DNA molecule and have proven useful in mapping genes and detecting single base-pair substitutions. We have determined the levels of intra-specific variation in the restriction maps of three species.

FS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron microscope heteroduplex analysis and restriction endonuclease cleavage analysis are currently being used to estimate the nucleotide sequence divergence in the circular mitochrondrial DNAs of different species of Drosophila.

MAJOR FINDINGS AND PROPOSED COURSE: Size heterogeneity has been detected in the mitochondrial DNAs of distantly related species of Drosophila. Electron microscope partial denaturation mapping has revealed the presence of adenine-thymine (A-T) rich regions in the mitochondrial DNAs of Drosophila. The length of the AT-rich region varies in different species. Heteroduplex analysis has shown the AT-rich region to be the evolutionarily most pliable region in the mitochondrial DNA of Drosophila. Specific cleavage sites of four different restriction endonucleases have been mapped on mitochondrial DNAs of three species of Drosophila. Some important information has been obtained from the restriction endonuclease cleavage maps concerning the evolution of these mitochondrial DNAs. Population genetic variation in the restriction maps of three species has been determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Genetic variation in mitochondrial DNA of higher eukaryotic organisms must be understood in order to judge the consequences of mutational damage. Very little is known about the extent and nature of genetic variation in mitochondrial chromosomes of animals. This project provides some basic information and hopefully will provide some evidence for the role of newly arising mutants.

PUBLICATIONS

Shah, D.M. and Langley, C.H.: Electron microscope DNAs: Evolution of A + T-rich region. Plasmid 2: 69-78, 1979.

Shah, D.M. and Langley, C.H.: Inter- and intra-specific variation in restriction maps of Drosophila mitochondrial DNAs. Nature 281: 696-699, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60058-04 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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. Langley	Research Geneticist	LAG	NIEHS
Other:	R. Voelker	Senior Staff Fellow	LAG	NIEHS
	A.J. Leigh Brown	Visiting Fellow	LAG	NIEHS
	S. Ohnishi	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:
3.3	1.2	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)

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. 10-76)

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

Voelker, R. A., Ohnishi, S., and Langley, C. H.: Genetic and cytogenetic studies of four glycolytic enzymes in Drosophila melanogaster: Aldolase, Triosephosphate Isomerase, 3-Phosphoglycerate Kinase, and Phosphoglucomutase. Biochem. Genet. 17(7/8): 769-783, 1979.

Voelker, R. A., Ohnishi, S., and Langley, C. H.: Genetic and cytogenetic studies of the malate dehydrogenase of Drosophila melanogaster. Biochem. Genet. 17(9/10): 947-956, 1979.

Voelker, R.A., Langley, C.H., Leigh Brown, A.J., Ohnishi, S., Dickson, B., Montgomery, E., and Smith, S.C.: Enzyme null alleles in natural populations of Drosophila melanogaster: Frequencies in a North Carolina population. Proc. Natl Acad. Sci., USA 77(2): 1091-1095, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60059-04 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Chromosomal Aberrations on Embryonic Development

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

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Other:	R. Sorg	Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

H. Michelmann, University of Göttingen, West Germany

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Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The project is designed to study the effects of chromosomal aberrations on fetal mortality and development. Comparisons are made between in vivo effects and effects on mouse embryos grown in culture. Some chemical mutagens are known to be capable of inducing chromosomal aberrations such as translocations which may be transmitted to the next generation. Increased rates of fetal death are usually observed among offspring of translocation heterozygotes. It is our purpose to investigate the rates of transmission of such aberrations, and the processes of fetal mortality by direct observation of the development of embryos.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female offspring of known translocation bearing males were mated to normal males. Two cell embryos were collected from these matings and grown in culture to determine overall loss of embryos and stage of disruption of foetal development.

MAJOR FINDINGS AND PROPOSED COURSE: The daughters of translocation males fall into two classes: those which present normal development among their offspring, and those which show a high frequency of foetal death. It is postulated that these latter females represent those which have inherited the translocation from their sire.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chromosome aberrations in man are known to cause disturbances in development of embryos leading to death of the embryo or to abnormal offspring. An understanding of the fate and effects of chromosome aberrations which may be caused by environmental agents is necessary for evaluation of the possible risks such induced aberrations may imply for man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60062-04 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 Li	Research Geneticist	LAG	NIEHS
Others:	M. Okabe	Visiting Fellow	LAG	NIEHS
	Y.-C. Pan	Visiting Associate	LAG	NIEHS

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

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Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIH, NIEHS, Research Triangle Park, NC 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 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-gylcerol phosphate dehydrogenase isozymes from Drosophila larva and adult, have been determined.

PHS-6040
(Rev. 10-76)

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 eluted peptides were determined by automatic amino acid analyzer.

MAJOR FINDINGS AND PROPOSED COURSE: 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.

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.

PUBLICATIONS

Chang, S.-M.T., C.-Y. Lee and S. S.-L. Li: Structural relatedness of mouse lactate dehydrogenase isozymes, A4 (muscle), B4 (heart), and C4 (testis). Biochem. Genetics, 17: 715-729, 1979.

Chang, S.-M. T., C.-Y. Lee, and S. S.-L. Li: Some chemical properties of rat testicular lactate dehydrogenase. Int. J. Biochem., 11: 1-6, 1980.

Li, S.S.-L., Y. Nakashima, J. P. Marciniszyn, Jr., S.-M.T. Chang, Y.-C. E. Pan, and S. Huang: Structural comparison of mouse lactate dehydrogenase isozymes, A4 (muscle), B4 (heart), C4(testis). XIth Int. Congress Biochem., p. 210, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60087-03 LAG

PERIOD COVERED
October 1, 1979 to March 30, 1980

TITLE OF PROJECT (80 characters or less)
Immunology and Structure of Mouse PGK Isozymes and Genetic Variations

(Former Title: Biochemical Studies of Mouse Enzymes, II. PGK-B Variants)

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

PI:	Chi-Yu Lee	Group Leader	LAG	NIEHS
Others:	B. Pegoraro	Visiting Fellow	LMG	NIEHS
	D. Niesel	Student Employee	LAG	NIEHS

COOPERATING UNITS (if any)
Robert P. Erickson, Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

To isozymes of mouse 3-phosphoglycerate kinase (PGK) were purified to homogeneity by an 8-(6-aminohexyl)-amino-Sepharose column followed by an ion exchange and gel filtration chromatography. Antisera against two PGK isozymes were raised separately in rabbits. Immunological cross-reactivity between the PGK isozymes were investigated by double immunodiffusion and enzyme immuno-inactivation. Amino acid composition and peptide maps were determined for the two PGK isozymes to compare their structural homology.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) General ligand affinity chromatography was employed as a tool for enzyme purification. (2) Double immunodiffusion and enzyme immunoinactivation were employed to investigate the cross-reactivity between the two PGK isozymes. (3) Structural homology of two PGK isozymes was studied by amino acid composition and analysis of peptide mapping.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Immunologically, antiserum to PGK-2 (sperm-specific) does not cross-react with PGK-1, whereas a partial cross reactivity between antiserum to PGK-1 and PGK-2 was observed. (2) Structurally, X-linked PGK-1 and autosomal sperm-specific PGK-2 share structural homology by amino acid composition analysis. By peptide mapping, however, no obvious homology between PGK-1 and PGK-2 was observed. (3) PGK-2C, a low activity variant, in C57L/J mice have only 2% of activity compared to the wild type and was shown to result from a structural gene mutations that affect the active site of the enzyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Study of PGK isozyme system in mice is important for the understanding of X-chromosome inactivation during spermatogenesis. The results of our study seem to indicate that PGK-1 and PGK-2 are structurally and immunologically distinct but related. They have, however, identical biochemical properties. Biochemical study of PGK-2C, a low activity variant, is important to understand how a gene mutation could affect the sperm fertility in mice.

PUBLICATIONS

Lee, C.-Y., Niesel, D., Pegoraro, B., and Erickson, R.P.: Immunological and structural relatedness of isozymes and genetic variants of 3-phosphoglycerate kinase from the mouse. J. Biol. Chem. (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60092-03 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical Mutations in Drosophila Induced by Chronic Irradiation

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
Other:	R. Voelker	Senior Staff Fellow	LAG	NIEHS
	R. Racine	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:
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)

An analysis of mutants recovered after 1000 lines of a balanced lethal strain of Drosophila melanogaster were exposed to chronic low-dose (8.7 rad/hr) γ-irradiation was carried out. The following mutants were recovered: Got-2, 1; α-Gdph, 6; cMdh, 2; Adh, 0; Dip-A, 0; Hex-C, 4; α-Amy, 0. These represent 7 independent mutation events. Only one of these was associated with a chromosomal aberration (deficiency). Of five which could be analyzed for CRM production, three were CRM-positive. These results suggest that the majority of mutants induced by low-dose-rate γ-irradiation are point mutations.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A strain of Drosophila melanogaster composed of balanced lethal heterozygotes, SM1/+, was replicated into 1000 lines and brother-sister mated each generation while being exposed to low-dose-rate γ-radiation (8.7 rads/hour) for 15 generations (a cumulative dose of 43848 rads). This strain is heteroallelic for the soluble enzyme loci Got-2, α-Gdph, cMdh, Adh, Dip-A, Hex-C and α-Amy. When electrophoresed after the irradiation, the unmutated files give codominant phenotypes. If one of the alleles in a particular line has sustained a null mutation (loss of enzyme activity) the phenotype will appear dominant (or homozygous).

MAJOR FINDINGS AND PROPOSED COURSE: 13 mutations were found at four different loci: 6 α-Gdph, 4 Hex-C, 2 cMdh, and 1 Got-2. Seven of the mutants are of independent origin and are apparent nulls. Three mutants (α-Gdph, 2 cMdh) produced gene products and are probably point mutations. Four mutants, (2 α-Gdph, cMdh and Got-2) produced no functional hetero- or homodimers as detected from gel staining.

The mutant salivary gland chromosomes were also analyzed. Of the 7 independent mutants, none appear to be associated with a chromosome aberrations. One α-Gdph null does, however, appear to be associated with a genetic deficiency which is too small to be observed cytologically.

This experiment suggests that with chronic low-dose-rate irradiation, the proportion of cytogenetic deletions is low. In order to get an estimate of the mutation rate induced by chronic low dose rate γ-radiation, a recessive-lethal experiment was carried out for the X and 2nd chromsomes. One generation (from egg to adult fly) was chronically irradiated at a low dose rate (8.4 rads/hour) for 14 days (a cumulative dose of 2923 rads). Induced recessive lethal rates for the X and 2nd chromosomes of 3.7% and 11.7%, respectively, were found. This recessive lethal experiment indicates that chronic γ-radiation leads to a similar number of mutants per rad as acute irradiation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings suggest that the majority of mutations induced by chronic low dose-rate radiation are point mutations rather than chromosomal aberrations.

PUBLICATION

Racine, R.R., Langley, C.H. and Voelker, R.A.: Enzyme mutants induced by low-dose-rate γ-irradiation in Drosophila: Frequency and characterization. Environ. Mutagenesis (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60094-03 LAG

PERIOD COVERED
October 1, 1979 to March 31, 1980

TITLE OF PROJECT (80 characters or less)

BrdU-Dye Studies of Meiotic Recombination

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

PI:	J. Allen	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:
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)

In vivo 5-bromodeoxyuridine (BrdU)-dye methodology is utilized for a variety of cytogenetic studies in rodent germ cells. Analyses of spermatogonial DNA replication and sister chromatid exchange (SCE) patterns are accomplished, and meiotic exchanges are visualized in 1° and 2° spermatocytes. Together, these studies can form a system for interpreting details of meiotic recombination. Mutagen effects upon SCE and/or meiotic exchange will be examined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In vivo approaches are used in a rodent system to allow for detection of BrdU in various germ cell stages. Methods based upon administering BrdU by serial injections, or by tablet implantation, provide for continuous exposure of replicating spermatogonial cells to analogue. Detection of BrdU in spermatogonial or spermatocyte stages is accomplished with differential staining in accordance with Hoechst fluorescence or Hoechst + Giemsa techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Meiotic exchanges can be visualized in a mammalian system, and these can be studied in regard to various characteristics of crossing over. This study has been terminated due to the transfer of the Principle Investigator to another government agency. It will be picked up at a later date at the Environmental Protection Agency.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Meiotic recombination is a principle source of genetic variability; yet it is poorly understood at the cytological as well as the molecular level. Very little is known about possible drug effects on this process. Cytogenetic analyses of spermatocyte cross-over events, using high resolution BrdU-dye techniques, can provide new information regarding germ cell DNA synthesis and exchange. In a mammalian system, this approach can be used to examine possible drug effects on SCE and meiotic exchange processes.

PUBLICATIONS

Allen, J.W., Shuler, C.F., and Latt, S.A.: Bromodeoxyuridine tablet methodology for in vivo studies of DNA synthesis. Som. Cell Genet. 4: 393-405, 1978.

Allen, J.W.: BrdU-dye characterization of late replication and meiotic recombination in Armenian hamster germ cells. Chromosome 74: 189-207, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60095-03 LAG

PERIOD COVERED
October 1, 1979 to March 31, 1980

TITLE OF PROJECT (80 characters or less)

Sister Chromatid Exchange Analyses in Rodent Fetal Tissues

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

PI:	J. Allen	Research Biologist	GTD	EPA
Other:	M. Sanyal	Reproductive Physiologist	LRDT	NIEHS
	E. El-Nahass	Visiting Fellow	LOFT	NIEHS
	R. Sharma	Visiting Fellow	LAG	NIEHS
	B. Gladen	Statistician	BB	NIEHS

COOPERATING UNITS (if any) Genetic Toxicology Division, Health Effects Research Laboratory, EPA; Laboratory of Research and Developmental Toxicology; Laboratory of Organ Function and Toxicology; Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.7	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 vivo sister chromatid exchange (SCE) analyses have been extended to rat fetal as well as maternal tissues. SCE induction effects from selected mtagens/teratogens are being examined after maternal drug metabolism and transplacental passage to various fetal tissues. In some instances, in vitro methodology is also employed in order to study direct effects from the parent compound, and also from known metabolites. The system should allow for sensitive cytological assessment of comparative drug damage at various maternal-fetal interaction stages.

HHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Subcutaneous BrdU tablet implantation in pregnant rats is used to effect analogue substitution in maternal and fetal tissues. Sister chromatid differentiation and exchange analysis is then carried out after Hoechst or Hoechst-and-Giemsa staining of chromosome preparations from various tissues. SCE increments are detectable after drug exposures during in vitro or in vivo DNA replication periods.

MAJOR FINDINGS AND PROPOSED COURSE: Experimental conditions have been worked out for effecting sister chromatid differentiation in multiple fetal tissues within an in vivo setting. The system, inclusive of in vitro components, permits assessment of directing drug damage, or damage after various physiological influences in regard to metabolism and placental transfer of the drug. Due to the transfer of the Principle Investigator from NIEHS to EPA, this project is being continued through collaborative efforts, under the direction of the Principle Investigator at EPA. The project will become a collaborative effort on the part of NIEHS in the future, with primary funding through EPA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In vivo analyses of SCE permit sensitive detection of genetic damage by drugs subject to host activation or detoxification. Comparative studies of SCE induction in maternal and fetal tissues should affort an estimation of tissue-specific sensitivity to the test drug. Various mutagens/teratogens with action at critical fetal development stages may be examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60096-03 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparison of Rates of Induction of Point Mutations and Chromosome Aberrations in Cultured Cells

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

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Other:	R. Sorg	Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

D. Clive, Burroughs Wellcome, Research Triangle Park, North Carolina

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

In order to assess the utility of various test systems, different aspects of their potential should be explored. If one type of genetic damage is more easily detected within a system, this might be the criterion of choice. Using the Mouse Lymphoma cell line L5178Y, mutations at the TK locus were induced by chemical treatment. Studies utilizing the same mutagens are conducted on the frequencies of induction of chromosomal damage and aberrations, and compared with the point mutation frequency.

FORM 2040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: L5178Y mouse lymphoma cells are cultured according to the methods developed by Clive and Spector (1975). Chemical treatment was with EMS (Ethylmethanessulfonate) at various dose levels. TK locus mutation frequencies are determined according to criteria given by Clive, et. al. (1979). Cells were prepared for cytogenetic analysis by treatment with colchicine for 2 hours followed by hypotonic treatment and fixation. Preparations were stained with giemsa.

MAJOR FINDINGS AND PROPOSED COURSE: Determinations to date of the frequencies of genetic damage indicate that there is a correlation between the induction of point mutations and the induction of chromosomal aberrations. Whether this correlation is coincidental or whether there is a real relationship is currently being considered. Studies include cytogenetic analysis of specific mutant cell lines recovered in the point-mutation studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The need for efficient biological detectors of genetic effects from environmental mutagens can be filled only when the detection system has been validated. Such validation can, to some extent, be arrived at by using several different genetic end points, and comparing their efficiencies, so that information regarding the basic mechanisms and relationships underlying induction of genetic damage may be deduced.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60097-01 LAG

PERIOD COVERED
October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Detection of Recessive Lethal Mutations Induced by Chemicals in Female Mice

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

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Others:	R. Dunn	Biologist	LAG	NIEHS
	R. Sorg	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:
2.1	0.8	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)

Studies are being conducted on the detection of Recessive Lethal Mutations induced in the germ cells of female mice by chemical mutagens. Females were treated at various ages of foetal development and their individual male offspring are studied. An increase in foetal mortality in backcrosses between a male and his daughters is taken as an indicator of the presence of a recessive lethal. Few studies of mutation induction in female germ cells by chemicals have been conducted, and none have utilized early germ cell stages. It is our purpose to determine if mutations can be induced in these stages, and at what frequencies they occur.

PHS-1040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant female mice of the inbred CBA/ca strain were treated with a known chemical mutagen (TEM) at either the 13th or 18th day of pregnancy. Female progeny were collected, and at maturity were bred to produce F_1 male offspring. The individual F_1 males are being tested in a series of backcrosses with their F_2 daughters to determine whether there is an increase in foetal mortality indicative of the presence of a recessive lethal.

MAJOR FINDINGS AND PROPOSED COURSE: The F_1 males are still under study, however, preliminary results indicate that a proportion of males are carrying recessive lethals. It is proposed to study individual mutations in an attempt to determine the time and mode of action of these genes in early developmental stages of homozygotes. Future attempts at biochemical identification of the loci in question will be made.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is estimated that on the average the human genome contains 5 deleterious recessive mutations. Although there are known examples of genes which have an extreme effect in the homozygote also showing an effect in the heterozygote, for the most part little is known about the mode of action or long-term effects on the bearer of such genes. Studies are necessary to determine frequencies of induction of this class of mutations by mutagenic agents and their consequences to the individual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60098-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 Li	Research Geneticist	LAG	NIEHS
Others:	Y.-C. Pan	Visiting Associate	LAG	NIEHS
	M. Okabe	Visiting Fellow	LAG	NIEHS
	F. Sharief	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:
2.3	2.0	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)

Lactate dehydrogenase isozymes, purified from mouse muscle (M), mouse heart (H), mouse testis (X) and rat testis (X), have been cleaved into small peptides by CNBr and trypsin. Most of these peptides have been separated and their amino acid sequences determined. Thus far, 95% of mouse LDH-X, 50% of mouse LDH-M, 10% of mouse LDH-H and 20% of rat LDH-X (330 amino acids in each protein) have been sequenced.

(Rev. 10-76)

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: The amino acid sequences of 95% of the 330 residues from mouse testicular LDH-X, 50% of mouse LDH-M, 10% of mouse LDH-H and 20% of rat LDH-X have been determined. The complete covalent structure of these LDH isozymes will be established, and their submolecular fragments will also be used to study the antigenic structure of this enzyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THIS INSTITUTE: The amino acid sequence differences among various LDH 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., S. Huang, J. P. Marciniszyn, S.-M.T. Chang, C.-Y. Lee and S.S.-L. Li: Amino acid sequence studies on sperm-specific lactate dehydrogenase isozymes from mouse and rat. Environ. Hlth Perspect., (in press).

Pan, Y.-C.E., S. Huang, J. P. Marciniszyn, Jr., C.-Y. Lee, and S.S.-L. Li: The preliminary amino acid sequence of mouse testicular lactate dehydrogenase. Hoppe-Seyler's Z. Physiol. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60099-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Protein-Nucleic Acid Interactions and Chromatin Structure-Function

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

PI:	Steven Li	Research Geneticist	LAG	NIEHS
Others:	Y.-C. Pan	Visiting Associate	LAG	NIEHS
	Y. Nakashima	Visiting Scientist	LAG	NIEHS
	F. Sharief	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:
1.2	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)

Approximately 85% of the 333 residues from the gene 32 DNA-binding protein of bacteriophase T4 has been sequenced. The complete primary structure of the major coat proteins from single-stranded DNA viruses If1 and Ike have also been determined.

FSS-6040
(Rev. 10-76)

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.

MAJOR FINDINGS AND PROPOSED COURSE: The amino acid sequences of approximately 85% of the 333 residues from T4 gene 32 protein and the complete sequences of coat proteins from If1 and Ike viruses have been determined. The unique characteristics of their amino acid sequences are being correlated with their functions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chemical structure of the DNA-interacting proteins, namely, T4 gene 32 protein and coat proteins of If1 and Ike viruses, 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.

PUBLICATIONS

Nakashima, Y., S.S.-L. Li, M. Setoguchi, K. R. Williams and W. H. Konigsberg: Structural studies of the T4 gene 32 protein. XIth Int. Congress Biochem., p. 40, 1979.

Nakashima, Y., and S.S.-L. Li: Primary structure studies on major coat proteins of DNA filamentous viruses and on gene 32 protein of T4 phase. Environ. Hlth Perspec. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61000-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Transplacental Induction of Sister Chromatid Exchanges in Developing Rat Embryos by 4-Nitroquinoline 1-oxide and Procarbazine.

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

PI:	Rajendar K. Sharma	Visiting Fellow	LAG	NIEHS
Others:	Ronnie Dunn	Biologist	LAG	NIEHS
	James Allen	Research Biologist	GTD	EPA

COOPERATING UNITS (if any)
Genetic Toxicology Division, Health Effects Research Laboratory, EPA

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	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)

Sister chromatid exchanges have been used as a sensitive indicator of mutagenesis. In this project, in vivo BrdU methodology is used to detect SCE induction in rat embryos when the pregnant mothers are exposed to known carcinogens 4NQO and Procarbazine. The purpose of these experiments is to explore the use of SCEs in assessing transplacental genetic damage caused by environmental mutagens/carcinogens/teratogens.

FORM HHS-3040

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant rats are subcutaneously implanted with a BrdU tablet and subsequently injected ip with 4-Nitroquinoline 1-oxide or Procarbazine. Tissues of embryo are harvested when the cells have undergone two cycles of division in the presence of BrdU. Chromosome preparations are made using standard techniques and slides are stained with Hoechst 33258. Fluorescence microscopy is used for visualizing chromatid differentiation and SCEs. Liver and yolk sac are examined to compare their relative sensitivities and liver is harvested at different timings to determine the effect of age of embryo on its susceptibility to genetic damage caused by environmental agents. SCEs in maternal bone-marrow cells are also examined for the purpose of comparison with SCEs induced in embryo tissues.

MAJOR FINDINGS AND PROPOSED COURSE: Treatment of pregnant mothers with 4NQO indicated a significant induction of SCEs in its embryo's liver at 14 days of gestation. A significant dose response with increasing dose is observed. The results of induction by 4NQO in embryo liver at day 17 of gestation are being analyzed. Experiments with Procarbazine are still in progress. Similarly, experiments on induction of SCEs in yolk sac with 4NQO and Procarbazine are also in progress. In future similar experiments will be performed with other mutagens/carcinogens/teratogens to verify the results of current experiments. The approach will be extended to include several other tissues of embryos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the increased exposure of man to chemicals and environmental pollutants, it is important not only to determine the genetic damage caused to persons exposed to these agents but also to assess the effects on the next generation. These experiments are designed to determine the degree of mutagenicity in progeny if pregnant mothers are exposed to chemicals. These experiments are in line with the Institute's objective of finding ways to assess the damage caused by various environmental agents so that the human health can be protected by avoiding harmful chemicals, etc.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61001-02 LAG

PERIOD COVERED
October 1, 1979 to March 31, 1980

TITLE OF PROJECT (80 characters or less)

Sister Chromatid Exchange Studies in Dividing Cells of the Rodent Colon.

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

PI:	G. Roberts	Visiting Fellow	LAG	NIEHS
Other:	J. Allen	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:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We propose to develop an in vivo system which would enable the examination of sister chromatid exchanges (SCE) in cells of the colonic epithelium of rodents. Methodology needs to be worked out so that dividing cells from deep within the Crypts of Lieberkuhn can be obtained in sufficient numbers. 5-Bromodeoxyuridine (BrdU)-dye techniques will be used to visualize both baseline and drug-induced SCE.

HHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A number of physical and chemical manipulations are being tried in order to determine the conditions which produce the greatest number of the target cells. Following this, various procedures for effecting BrdU incorporation will be tested and the timing of administration of the analogue will be varied. Hopefully, a method for obtaining good numbers of metaphase cells showing sister chromatid differentiation will result.

MAJOR FINDINGS AND PROPOSED COURSE: Experimental conditions have been worked out which do permit the release of many dividing cells. To visualize sister chromatid differentiation, BrdU will be administered by various routes and at various times prior to harvest of cells. Due to the transfer of the Principle Investigator and other individuals involved, this project has been terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tumors of the colon are one of the most common forms of neoplasia in the Western World, and in fact, several agents specifically induce colonic tumors. Development of the techniques mentioned here may provide a model for the study of the kinds of lesions induced by carcinogens in the gastro-intestinal tract. It may also be possible to study the kinetics of cell division in the intestine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61002-02 LAG

PERIOD COVERED
October 1, 1979 to March 31, 1980

TITLE OF PROJECT (80 characters or less)

Induction of Sister Chromatid Exchanges (SCE) by Ethyl Carbamate in Various Mouse Tissues

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

PI:	G. Roberts	Visiting Fellow	LAG	NIEHS
Other:	J. Allen	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:
0.8	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)

Differences in baseline and drug-induced frequencies of SCE between somatic and germ cells have been demonstrated. This capability of the SCE test was utilized to determine the relative sensitivity of various mouse tissues to the carcinogen ethyl carbamate. 5-Bromodeoxyuridine (BrdU)-dye methodology was utilized for examining SCE in vivo and in vitro. The extent of SCE induction in the tissues used correlates well with their known sensitivity to this agent with respect to the induction of carcinogenic and mutagenic endpoints.

PROJECT DESCRIPTION

METHODS EMPLOYED: Cells from mouse bone marrow, liver and spermatogonia were examined for SCE induction by ethyl carbamate. 5-Bromodeoxyuridine (BrdU) was incorporated into the chromosomes of the former two tissues by the subcutaneous implantation of a slowly dissolving tablet of the analogue. Recently hepatectomized mice were used if liver was to be studied. The incorporation of BrdU into spermatogonial cells was effected by means of a series of intraperitoneal injections. In addition, bone marrow cells were cultured in the presence of BrdU in order to confirm the negative effects of ethyl carbamate in vitro.

MAJOR FINDINGS AND PROPOSED COURSE: Each of the tissues responded to ethyl carbamate exposure in vivo with a dose-dependent increase of SCE; however, there were large discrepancies in the extent of this response. Ethyl carbamate is widely and evenly distributed in the mouse; therefore, variable metabolism between tissues or differential sensitivity to the active compound most likely accounts for the observed effects. The tissue responses in order of decreasing sensitivity were as follows: liver, bone marrow, and spermatogonia. Previous negative reports for SCE induction in vitro were confirmed. Further studies with agents of different types and mechanisms of action are required to fully understand the relevance of differential tissue sensitivities. Due to the transfer of both the Principle Investigator and other coworkers, this study has been terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The analysis of SCE induction in vivo is a highly relevant test system as the metabolic, distribution and excretory systems of the host are intact. This study exemplifies the greater usefulness of in vivo testing in a number of tissues as compared to in vitro studies. Together with further studies of this nature, we may learn more about the factors involved in differential tissue sensitivities to carcinogen/mutagen insult.

PUBLICATIONS

Roberts, G.T. and Allen, J.W.: Tissue-specific induction of sister chromatid exchanges by ethyl carbamate in mice. Environmental Mutagenesis (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61003-02 LAG

PERIOD COVERED
October 1, 1979 to March 30, 1980

TITLE OF PROJECT (80 characters or less)
Analysis of Natural and Induced Null Variants of α-GPD in Drosophila

(Former Title: Biochemical Analyses of Drosophila Null Mutants)

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	LAG	NIEHS
Others:	D. Niesel	Student Employee	LAG	NIEHS
	S. Read	Student Employee	LAG	NIEHS
	L. Johnson	Student Employee	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:
0.2	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

More than two dozen of null mutants of α-glycerol phosphate dehydrogenase have been identified in natural and γ-induced or EMS-treated Drosophila populations. To understand the nature of gene mutation in α-Gdph locus, we have analyzed these null mutants by double immunodiffusions, rocket immunoelectrophoresis and two-dimensional electrophoresis. One out of nine mutants identified in Drosophila natural populations was shown to be CRM-negative. One out of four mutants identified in γ-irradiated Drosophila was shown to be CRM-positive. All the EMS-induced null mutants were shown to be CRM-negative.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) α-glycerol phosphate dehydrogenase was purified by affinity chromatography using an 8-(6-aminohexyl)-amino-ATP-Sehparose column and antisera against purified enzyme were raised in rabbits. (2) Rocket immunoelectrophoresis was employed as a tool to estimate the amount of cross-reacting materials (CRM) in mutants. Qualitatively, the mutants were also analyzed by double immunodiffusion and two-dimensional gel electrophoresis. Consistency has been observed among the results obtained from these analytical methods.

MAJOR FINDINGS AND PROPOSED COURSE: In this analysis, we attempted to understand why α-Gdph locus exhibits highest mutation frequency/locus either in natural or γ-irradiated Drosophila populations. Majority of the null mutants identified in natural population seems to be CRM-positive, whereas those identified in γ-irradiated lines are CRM-negative. Further study with recombinant DNA technique is essential to elucidate the exact nature of gene mutation at DNA levels.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The present study is important for the understanding of the nature of gene mutations as well as the processes of environmental mutagenesis. α-Gdph is an important biochemical marker for the mutation screening of environmental mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61004-02 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Senior Staff Fellow	LAG	NIEHS
	A. Leigh Brown	Visiting Fellow	LAG	NIEHS
	R. Racine	Visiting Fellow	LAG	NIEHS
	S. Ohnishi	Visiting Fellow	LAG	NIEHS
	S. Smith	Student	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:
2.6	2.3	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)

Two-dimensional electrophoresis as described by O'Farrel in 1975 has been applied to the identification and estimation of genic 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. 10-76)

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 with some investigations of rates of molecular evolution in Drosophila and mice.

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

Leigh Brown, A.J. and Langley, C.H.: Reevaluation of level of genic heterozygosity in natural population of Drosophila melanogaster by two-dimensional electrophoresis. Proc. Nat'l Acad. Sci., USA 76: 2381-2384, 1979.

Racine, R.R. and Langley, C.H.: Genetic analysis of protein variation in Mus musculus using two-dimensional electrophoresis. Biochem. Genet. 18: 185-197, 1980.

Racine, R.R. and Langley, C.H.: Genetic heterozygosity in a natural population of Mus musculus assessed using two-dimensional electrophoresis. Nature 283: 855-857, 1980.

Smith, S.C., Racine, R.R. and Langley, C.H.: Two-dimensional electrophoretic survey of genetic variation in man. Genetics (in press).

Leigh Brown, A.J. and Langley, C.H.: Abundant proteins in Drosophila melanogaster adults: An analysis by two-dimensional electrophoresis. Biochem. Genet. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61005-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Genetic Analysis of RNA Polymerase II of Drosophila melanogaster

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

PI:	R. A. Voelker	Senior Staff Fellow	LAG	NIEHS
Others:	S. Ohnishi	Visiting Fellow	LAG	NIEHS
	B. Dickson	Biological Laboratory Technician	LAG	NIEHS
	S. M. Huang	Biological Laboratory Technician	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
NIH, NIEHS, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 of which confers α-amanitin resistance, has been identified. This locus will be used as a toe-hold to identify the loci coding for the other subunits by means of genetic interactions between this and the other loci.

PHS-6040
(Rev. 10-76)

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 α-amanitin-resistance; (2) modification of visible phenotypes known to be conditioned by the α-amanitin-resistance allele; (3) non-allele 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 other loci. We have recovered several temperature-sensitive lethal alleles at this locus and plan to screen for suppressors of the temperature-sensitive phenotype. The allele conferring α-amanitin resistance also acts as a dramatic enhancer of Ubx; we will screen for non-allelic enhancers and suppressors of the enhanced Ubx phenotype. In addition, the allele effecting α-amanitin resistance in certain combinations results in male sterility; the restoration of male fertility by mutations at other loci can be an effective screen for mutants of other subunits.

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

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

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61006-01 LAG

PERIOD COVERED
March 4, 1980 to September 30, 1980

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

PI:	P. Bingham	Staff Fellow	LAG	NIEHS
Other:	B. 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:
0.58	0.39	0.19

CHECK 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 demonstrated that, among a large array of Drosophila melanogaster strains (including those bearing various mutant alleles at the white locus), only those bearing the w^a allele are labeled at the cytological location of the white locus after in situ hybridization using as probe a cloned copy of an interspersed, reiterated DNA sequence. Most importantly a $w^{Bwx}w^a$ double mutant (w^{Bwx} is a white mutant allele mapping to the left of w^a) and a w^aw^{ch} double mutant (w^{ch} is a white mutant allele mapping to the right of w^a allele) are labeled at white in this way while neither the w^{Bwx} nor the w^{ch} allele alone is labeled. These results suggest that the w^a mutation results from the insertion of a copy of the cloned DNA sequence element into the white region DNA sequences.

FORM 7-3040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: These studies employ classical genetic and cytogenetic techniques, DNA cloning techniques and *in situ* hybridization.

MAJOR FINDINGS AND PROJECTED COURSE: Our results to date suggest that the w^a mutation at the white locus of *Drosophila melanogaster* results from the insertion of a new DNA sequence element into the white region DNA sequences. We plan to refine our analysis of this mutation by isolating newly generated recombinants between the w^a allele and other alleles at the white locus. The newly isolated recombinants will be assessed for the presence of the DNA sequence element copy by *in situ* hybridization. In addition we will examine a series of recombinants isolated by Judd (unpublished observations) and involving the w^a allele and various characterized genetic markers within the white locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The DNA sequence element we hypothesize to be responsible for the w^a mutation is a member of the class of interspersed, reiterated DNA sequence elements. These elements appear to be transposable and may account for a substantial fraction of spontaneous mutations in Drosophila. The study of these elements is expected to contribute significantly to our understanding of spontaneous mutation in metazoans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 61007-01 LAG

PERIOD COVERED
March 4, 1980 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Biochemical and Genetic Analysis of Regulation of White Locus Transcription

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

PI: P. 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:
0.38	0.19	0.19

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)

Genetic studies to date have defined the properties of a dominant mutant allele (w^{DZL}) at the white locus of Drosophila melanogaster. On the basis of the results of these studies I have concluded that this allele causes the repression of transcription of the white locus and that this allele exerts its effects in both the cis and trans configurations. We have recently cloned DNA sequences from the white locus region. In situ hybridization analysis to date demonstrates that the cloned sequences reside very near and probably within the region containing the white locus genetic elements.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: These studies employ classical genetic and cytogenetic techniques, DNA cloning techniques, restriction endonuclease cleavage site mapping techniques, electron microscopy and nucleic acid hybridization and heteroduplexing techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Genetic analysis has defined a number of mutant alleles that effect the regulation of expression of the white locus. The particular properties of these mutants alleles render the white locus a very exceptionally attractive experimental system for the biochemical analysis of the regulation of transcription in metazoans. I will pursue a detailed analysis of the transcripts homologous to the cloned white locus DNA sequences and originating in individuals of the appropriate genotypes. It is anticipated that these studies will lead to substantial refinement of extant mechanistic models for the regulation of white locus expression.

SIGNFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Understanding, in precise mechanical detail, the methods whereby gene expression is controlled in developing multicellular organisms is a central objective of contemporary molecular biology. Studies outlined here are expected to contribute substantially to the achievement of this objective. Apart from the critical importance of understanding the phenomena in question to the further development of a comprehensive theory of the functioning of organisms, progress in this area will have at least one important implication germaine to the program of the Institute: A detailed understanding of the functional organization of the metazoan genetic locus will materially enhance our capacity to assess the hazards associated with any particular mutagen on the basis of its observed mutagenic propensities.

PUBLICATIONS

Bingham, P.M.: The regulation of white locus expression: A dominant mutant allele at the white locus of Drosophila melanogaster. Genetics (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61008-01 LAG

PERIOD COVERED
March 4, 1980 to September 30, 1980

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:	P. Bingham	Staff Fellow	LAG	NIEHS

CODPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.38	0.19	0.19

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Genetic analysis has shown that a newly isolated dominant, mutant allele (w^{DZL}) at the white locus of *Drosophila melanogaster* is highly mutable and produces sequence rearrangement mutations of all varieties predicted by a transposable-element hypothesis for the physical basis of this mutable allele. We have recently cloned DNA sequences from the white locus region. In situ hybridization analysis demonstrates that the cloned sequences reside very near and probably within the region containing the white locus genetic elements. These same data corroborate the results of cytogenetic analysis mentioned above and thus support the transposable-element hypothesis.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: These studies employ classical genetic and cytogenetic techniques, DNA cloning techniques, restriction endonuclease cleavage site mapping techniques, electron microscopy and nucleic acid hybridization and heteroduplexing techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Genetic and cytogenetic analyses demonstrate that sequence rearrangement mutations generated by a mutable allele at the white locus (w^{DZL}) share a common breakpoint and that this breakpoint corresponds to the locus of the mutable mutation. These observations strongly support the hypothesis that this mutable mutation arose as the result of the insertion of a new DNA sequence element (into the white region DNA sequences) possessing properties similar to those of the well characterized transposable elements in bacterial systems. Initial results with the newly cloned white locus DNA sequences corroborate the positioning of the breakpoints for these rearrangement mutations originally performed on the basis of classical analyses. I plan to examine in detail the physical structure of several mutable alleles at the white locus and of the rearrangement variants produced by these alleles. It is anticipated that these studies will contribute materially to our understanding of the mutational processes involved.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: A number of extant observations suggest that transposable DNA sequence elements are a major source of spontaneous mutations in metazoans. A detailed description for these elements and their capacity to mediate mutation is of first importance to an understanding of mutation in metazoans.

PUBLICATIONS

Bingham, P.M.: The regulation of white locus expression: A dominant mutant allele at the white locus of Drosophila melanogaster. Genetics (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61009-01 LAG

PERIOD COVERED
October 1, 1979 to March 30, 1980

TITLE OF PROJECT (80 characters or less)

Genetic Variants of Phosphoglucose Isomerase from Mouse and Drosophila

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

PI:	Chi-Yu Lee	Group Leader	LAG	NIEHS
Others:	Daniel Charles	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

Robert Voelker, Laboratory of Animal Genetics, NIEHS

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

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

Genetic variants of phosphoglucose isomerase were purified from mouse and Drosophila by 8-(6-aminohexyl)-amino-ATP-Sepharose-column followed by preparative isoelectric focusing. Antisera were raised for purified mouse and Drosophila enzymes in rabbits. A null variant of phosphoglucose isomerase identified in natural populations of Drosophila was shown to produce no proteins that cross-react with the antiserum specific to this enzyme (CRM-negative).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) General ligand affinity chromatography was employed as a tool for enzyme purification. (2) Rocket immunoelectrophoresis was used as a tool to analyze the null mutants in heterozygous state.

MAJOR FINDINGS AND PROPOSED COURSE: A null mutant of Drosophila Phosphoglucose Isomerase was shown to be CRM-negative. The mutation can be of structural or regulatory origin. To investigate the nature of gene mutation of a recombinant DNA technique is essential to answer this problem.

SIGNIFICANCE OF BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The investigation of the nature of enzyme null mutation is important for the understanding of environmental mutagenesis, especially the mechanistic difference of spontaneous and induced gene mutations.

PUBLICATIONS

Charles, D., and Lee, C.-Y.: Biochemical characterization of phosphoglucose isomerase and genetic variations from mouse and Drosophila melanogaster. Mol. Cell Biol. 29: 11-21, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61010-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
Others:	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:
0.75	0.25	0.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The 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 aberration 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.

FS-6040
(Rev. 10-76)

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 25 kb. 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.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 61011-01 LAG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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.

FS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

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

MAJOR FINDINGS AND PROPOSED COURSE: We have concentrated on two loci in Drosophila that show different aspects of allelic interaction. The white locus exhibits some unusual characteristics in its regulation when homologous X chromosomes are paired vs. unpaird. 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 putative rearrangements have been recovered and are being characterized.

The proposed course for this study is to carry the analysis of the allelic interactions to the molecular level. The white locus has been cloned (see Z01 ES 61008-01 LAG) and its transcription product(s) will be sought. Analysis of mutants that upset the allelic interaction will be done in terms of the molecular structures of the mutant gene compared to normal. We will seek the mechanism by which the white locus interacts with the zeste locus product to produce repression of paired alleles.

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

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 α and β 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. V. Charles-Shannon; Pharmacologist - Dr. J.-S. Hong; Visiting Scientist - Dr. P. K. Seth; Psychologist - Dr. L. L. Uphouse; Technician - D. L. Maxson; Technician - R. L. McLamb. Individuals leaving the Laboratory of Behavioral and Neurological Toxicology were technicians W. C. Byrd and R. R. Rhoderick.

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; Contributor to Panel, "Animal Rights in Brain Research," Winter Conference on Brain Research, Keystone, Colorado; Invited presentation entitled "Experimental Opportunities with Chick Models in Brain Research," Winter Conference on Brain Research, Keystone, Colorado; Invited seminar entitled "Rapid Screening Through the Use of Short-Term In Vivo and In Vitro Techniques," FDA Symposium on the Effects of Food and Drugs on the Development and Function of the Nervous System, Washington, D. C.; Invited seminar entitled "Neurotransmitter Binding - Practical Applications," UNC Neurobiology Seminar Series; Invited seminar entitled "Use of Receptor in Neurotoxicological Studies," UNC Pharmacology Graduate Seminars.

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 "Species Comparisons in Behavioral Toxicology," Symposium on Behavioral Toxicology, Carolinas Conference on Psychology, Meredith College, Raleigh, North Carolina.

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; Chairperson, Animal Experimentation Committee, NIEHS; Chairperson, Ad Hoc Committee to Select CMB Chief, 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 "Improvement of Methods for the Study of the Effect of Environmental Factors on the Central Nervous System and Behavior: A Perspective," 3rd Joint US/USSR Symposium on Environmental Health, Suzdal, Russia, USSR; Invited seminar entitled "Screening for Neurobehavioral Toxicity," 1979 Annual DRUSAFE Meeting, Sponsored by the Pharmaceutical Manufacturing Association, Sea Island, Georgia; Invited presentation entitled "Overview of the Neurobehavioral Effects of Electromagnetic Waves," Winter Conference on Brain Research, Keystone, Colorado; Invited seminar entitled "Overview of the Research in Neurobehavioral Toxicology at NIEHS," 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; Member, Project Advisory Group, Project to Standardize and Validate Methods and Procedures in Behavioral Teratology, sponsored by the National Center for Toxicological Research, Jefferson, Arkansas; Member, Advisory Group to the Office of Toxic Substances to assess proposals to standardize behavioral tests in neurotoxicology, Washington, D. C.; Invited seminar entitled "The Neurotoxicology of Acrylamide - Evidence for Alterations in Brain Dopamine Function," Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90003-03 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Developmental Exposure to Polychlorinated Biphenyls on Neurobehavioral Functioning of Mice

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:	G. W. Lucier	Acting Chief	LOFT	NIEHS
	A. K. Agrawal	Visiting Fellow	LBNT	NIEHS
	S. C. Bondy	Head, Neurochemical Workgroup	LBNT	NIEHS

COOPERATING UNITS (if any)
Laboratory of Organ Function and Toxicology, NIEHS
Raltec Scientific Services, Madison, Wisconsin

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)

The purpose of these experiments is to determine the neurotoxic effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl (TCBs) in mice. Offspring of mothers given TCBs on days 6-13 of gestation are being assessed for neurological deficits for up to one year after birth.

FHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: When tested at adulthood, albino mice exposed to 3,4, 3',4'-tetrachlorobiphenyl (TCB) during gestation demonstrated signs of neurotoxicity. The most severely affected subjects (TCB-Spinners) displayed a neurobehavioral syndrome consisting of intermittent stereotypic circling, head-bobbing, and hyperactivity. TCB-Spinners were found to be markedly hyperactive during the dark phase of the diurnal phase and showed impaired forelimb grip strength, ability to traverse a wire rod, visual placement responding, and acquisition of one-way avoidance. Some mice did not display the spinning syndrome (TCB-Nonspinners) but were found to be deficient in traversing a wire rod and avoidance acquisition. None of the TCB-exposed mice were found to have depressed neuromuscular reflexes in response to a variety of stimuli. Tissue distribution studies demonstrated that TCB levels were not detectable in adult mice following prenatal exposures. The results of these experiments demonstrate that prenatal exposure to TCB 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.

Subsequent experiments with mice exposed to TCB have focused on the dopaminergic system. TCB-exposed mice were found to be less sensitive to the motor activity-decreasing effects of haloperidol. The corpous striatum, a dopamine rich area in the brain believed to be involved in the modulation of some types of motor activity, was found to have fewer dopamine receptors in TCB-exposed mice than in control mice. These data suggest that developmental exposure to TCB might have interfered with the development of brain dopamine systems.

Additional experiments are planned in which dopaminergic function of TCB-exposed mice, especially those exposed to lower amounts of TCB during gestation, will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developmental neurotoxicological potential of TCBs has not been assessed systematically. The experiments described above will provide a long range evaluation of perinatal exposure to PCBs on neurobehavioral functioning. Some insight into the mechanism by which perinatally administered TCBs affects the nervous system might also be attained.

PUBLICATIONS

Tilson, H. A., Davis, G. J., McLachlan, J. A., and Lucier, G. W.: The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. Environ. Res. 18: 466-474, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90007-03 LBNT

PERIOD COVERED
April 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Neurobehavioral Toxicity of Neonatal Exposure of Rats to Diethylstilbestrol and Compounds with Potential Estrogenic Activity

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. A. Lamartiniere	Senior Staff Fellow	LOFT	NIEHS
	O. A. Meyer	Visiting Scientist	LRDT	NIEHS
	V. Charles-Shannon	Interagency Personnel Agreement	LBNT	NIEHS

COOPERATING UNITS (if any)
Laboratory of Organ Function and Toxicology, NIEHS
Laboratory of Reproductive and Developmental 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:
1.1	0.5	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)

Many environmentally prevalent chemicals have or are suspected of having effects on estrogen (i.e., kepone, DDT, PCBs). The presence of estrogen at a critical period during development is believed to be necessary for the differentiation of of sexual, morphological, and functional characteristics. It is suspected that environmental toxicants might influence sexually dimorphic behaviors by interfering with the presence of estrogen during this critical phase. The purpose of this research is to: (1) develop an animal model to assess effects on sexual differentiation using chemicals such as estrogen and testosterone that have known effects on sexually dimorphic characteristics and (2) employ the model to study environmental toxicants with potential estrogenic activity. Reproductive-based sexual behaviors, nonreproductive sexually dimorphic behaviors, and organ weights will be used in these experiments; sexually dimorphic liver enzyme function will also be studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Intact or neonatally castrated male albino rats of the Sprague-Dawley strain were given s.c. injections of propylene glycol (PG), diethylstilbestrol (DES), estradiol-17β (E_2), or testosterone propionate (TP) on days 2, 4, and 6 postpartum. Intact females given PG neonatally were tested for comparative purposes. The neonatal administration of DES and E_2 to intact pups attenuated or blocked the course of sexual differentiation. Gonadal development of the E_2- and DES-treated animals was affected significantly, and the adult body weights, grip strength scores, and emergence latencies of these animals were not different from intact females given PG. Except for a delay in gonadal development, neonatal administration of TP had no significant neurobehavioral effects. The adult body weights, grip scores, and emergence latencies of male pups castrated at birth were similar to those of female rats and these effects were not altered by neonatal administration of DES or E_2. Neonatal administration of TP appeared to block the effects of castration of body weights and grip strength scores but did not alter emergence latencies. Intact males given DES or E_2 and castrates given PG, DES, or E_2 did not differ significantly from intact females given PG neonatal. These data indicate that high levels of E_2 and DES 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.

Studies are currently underway in which reproductive sexual behaviors are being used to determine the developmental effects of environmental toxicants such as methoxychlor and DDT.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is a need to understand more fully the effects of perinatal exposure to diethylstilbestrol and other hormonal agents on neurobehavioral functions using reproductive and sexual behaviors. The ability to detect other, more subtle behavioral effects may prove useful in determining the presence of previously unknown or poorly described psychological and neurobehavioral deficits. In addition, such studies may prove useful in elucidating the mechanism and site of action of such agents.

PUBLICATION

Tilson, H. A. and Lamartiniere, C. A.: Neonatal exposure to diethylstilbestrol affects the sexual differentiation of male rats. Neurobehav. Toxicol. 1: 123-128, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90008-03 LBNT

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	Staff Fellow	LBNT	NIEHS
	T. A. Burne	Psychologist	LBNT	NIEHS
	P. A. Cabe	Senior Staff Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

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:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The study of the neurobehavioral toxicity of acrylamide has developed in three phases:

A. The first phase consisted of the use of acrylamide toxicity to assist in the standardization and validation of a battery of neurobehavioral screening tests. To this end, the following experiments have been done:

(1) Lethal dose. A lethal dose (LD50) of 251 mg/kg was found at 12 hours and 175 mg/kg at 168 hours.

(2) Acute dosing. Caudal motor dysfunction was observed in the hindlimb extensor response and inclined screen tests 12 hours after a single dose of 50-200 mg/kg. Hindlimb function recovered completely by 168 hours. No effect on forelimb strength was observed.

(3) Short-term repeated dosing. Repeated 10-20 mg/kg doses over four weeks produced hindlimb deficits at cumulative doses of 50-100 mg/kg. No forelimb dysfunction appeared. General motor activity was decreased after four weeks of dosing. Recovery of function was incomplete at two weeks after cessation of dosing.

(4) Longer term repeated dosing. Rats receiving 20 mg/kg had significantly lower body weights at 4-13 weeks of dosing. Significant decreases in hindlimb function were observed during 7-13 weeks of dosing, while motor activity was decreased at 10-13 weeks. Forelimb grip strength was not affected during the dosing period. In rats receiving 10 mg/kg, there were no effects during the dosing period.

At the end of the 13 weeks of dosing, signs of early fiber degeneration were observed in the peripheral nerves of all animals receiving 10 mg/kg of acrylamide, while the rats in the high dose group were found to have signs of moderate to severe acrylamide neurotoxicity. One week after cessation of dosing, fore- and hindlimb scores, spontaneous motor activity, and body weights were significantly decreased in the high dose rats. Five weeks after cessation of dosing, the acrylamide-treated rats did not differ from controls on neurobehavioral measures. Neuropathological examination showed signs of regenerating and remyelinating fibers in the animals previously exposed to acrylamide.

The neurobehavioral screening procedures used in these experiments show both sensitivity and specificity to the neurotoxic effects of acrylamide. These and other studies in our laboratory have proven beneficial in the development of a battery of tests to be used to assess the neurological and behavioral toxicity of many types of environmental factors.

B. The second phase of this project consisted of developing secondary level tests to study the neurotoxicity of acrylamide. The purpose of these experiments is to standardize and validate more sensitive but more cost-effective, behavioral tests for use in behavioral toxicological assessments.

(1) Methods to assess motor function. Male rats were dosed orally with acrylamide monomer (10 mg/kg) or with distilled water vehicle (1 ml/100 g). Doses were administered five days/week for three weeks. Rate of responding on a fixed-ratio 30 (FR30) schedule of reinforcement at two lever heights was recorded. Percent change in rate from predosing to dosing and from dosing to postdosing was analyzed for possible deficits on the high and low positioned lever. Responding on the lever in the high position was taken as a measure of hindlimb function.

Dosing with acrylamide produced decreases in response rate at the high lever position but not at the low lever position. In addition, post reinforcement pause times (PRP), the amount of time it took for the rat to lower itself from the high lever to the food cup and return up, significantly increased in acrylamide-dosed rats. There was significant recovery of function in the post dosing phase. No obvious signs of toxicity were observed. Through continued refinement of apparatus, the precise onset of neuromuscular toxicity due to repeated acrylamide dosing in the fore- and hindlimbs of rodents could be assessed. Studies to measure acrylamide-induced changes in the actual force applied to the manipulandum are being planned.

(2) Methods to assess sensory function. A psychophysical titration technique has been developed to measure pain thresholds in rats exposed to neurotoxicants. An operant titration procedure that provides relatively stable within session pain or reaction thresholds (0.15-0.25 mA) within a week of training has been developed. Rats are placed into plexiglas restraint tubes having a hole at one end through which the animal can poke its nose; a photobeam is broken and a response is counted. The tail of the animal is firmly held by a plexiglas plug and an electrode connected to a programmable titration shocker is attached to the tail. Conditioned nose poke responses are made after about 15 min. during the first 30 min. session. The titration procedure was found to be sensitive to the analgesic effect of morphine. Significant increases in the median shock level tolerated was observed after 3 mg/kg, while response rate was not altered. Dose-related increases in threshold after 6 and 9 mg/kg of morphine were associated with decreases in the rate of nose poking. p-CPA had no significant effects on thresholds at a time when brain serotonin is presumed to be selectively decreased. The technique offers several advantages in the study of chemical-induced alterations in pain or reactivity including rapidity of training, relatively short time to establish median shock thresholds, minimum involvement of motor components in the response, and sensitivity to a psychopharmacological tool.

In studies with acrylamide, rats were dosed daily with 10 mg/kg p.o. and tested in the titration procedure. No effects on pain thresholds were seen after two weeks of dosing. These data are consistant with clinical reports that acrylamide does not affect pain sensitivity. The titration procedure is being used to assess the somatosensory effects of neurotoxicants other than acrylamide.

C. The third phase of methods development and validation using acrylamide concerns the use of naturally occurring home cage behaviors. The purpose of these studies will be to determine the effects of chronic exposure to acrylamide on patterns of diurnal and noctural food and water consumption, as well as the spontaneous locomotor activity of rats in domiciliary cages. Attempts will be made to measure changes from expected baselines in each behavioral variable as a function of dose, length of exposure to acrylamide, age, and sex of rat.

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

Cabe, P. A., Tilson, H. A., Mitchell, C. L., and Dennis, R.: A simple recording of grip strength device. Pharmacol. Biochem. Behav. 8: 101-102, 1978.

Tilson, H. A., Mitchell, C. L., and Cabe, P. A.: Screening for neurobehavioral toxicity: The need for and examples of validation of testing procedures. Neurobehav. Toxicol. 1 (Suppl. 1): 137-148, 1979.

Meyer, O. A., Tilson, H. A., Byrd, W. C., and Riley, M. T.: A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav. Toxicol. 1: 233-236, 1979.

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. Elsevier/North-Holland, New York, 1980, pp. 265-294.

Tilson, H. A., Cabe, P. A., and Burne, T. B.: Procedures to be Used in the Routine Assessment of Neurotoxicity in Rodents: Effects of Acrylamide. In Spencer, P. S. and Schaumburg, H. H. (Eds.): Experimental and Clinical Neurotoxicology: A Textbook of Environmental Neurobiology. Baltimore, Maryland, Williams and Wilkins (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90009-03 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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.

PHS-6040
(Rev. 10-76)

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.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90011-02 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LBNT NIEHS
Other:	N. E. Carmichael	Visiting Fellow	LOFT NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
	T. A. Burne	Psychologist	LBNT 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:
1.3	0.5	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)
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.

FORM 6040
(Rev. 10-76)

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. The neurotoxicity of TET is being assessed in secondary behavioral tests.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90012-02 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LBNT	NIEHS
	C. Lamartiniere	Senior Staff Fellow	LOFT	NIEHS
	O. Meyer	Visiting Scientist	LRDT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Organ Function and Toxicology, NIEHS
Laboratory of Environmental 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:
0.6	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)

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. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The s.c. administration of 2-3.5 mg of MSG/g of body weight on postnatal days 1-5 was found to alter neurobehavioral parameters at 60 and 90 days of age. Increased latencies to emerge into a novel environment and elevated startle responses were observed in MSG-treated animals; changes in sensitivity to a painful thermal stimulus were also observed. Neuromuscular strength and tests for motor coordination were generally unaffected. Measures of spontaneous motor activity at 100-120 days of age showed that MSG rats had lower activity scores; MSG-exposed animals appeared to be more sensitive to the motor activity increasing effects of d-amphetamine (0.3-3 mg/kg, i.p.). MSG-treated males and females were also found to have elevated p-450 hepatic enzyme function; males had reduced GS-t while females had reduced histidase activities. Prolactin levels were not affected by MSG, but testosterone levels were decreased significantly in the males. Other neuroendocrine hormones are being assayed.

Additional studies are now underway to determine the effects of various concentrations of saline (0.9, 13, and 22% NaCl) on neurobehavioral functioning. In previous work, high concentrations of NaCl were found to mimic the effects of MSG, although to a lesser degree. Thus, the developmental neurotoxicity of MSG may in some part be related to the hyperosmolarity of the MSG solution.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90013-02 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LBNT	NIEHS
	J. A. Moore	Associate Director	RRP	NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	P. K. Seth	Visiting Scientist	LBNT	NIEHS
	V. Charles-Shannon	Interagency Personnel Agreement	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:
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)

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 neurochemcial effects of kepone.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Thus far, this project consists of two experiments:

A. Short term, dose ranging study (51 days). Male and female albino rats of the Fisher-344 strain weighing approximately 250 g at the start of the study received 0, 10, or 30 ppm kepone (K) 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 K 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 K (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 K 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 K, but males given 30 ppm K 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 K (133% of control) and increased retention latencies for males exposed to 10 and 30 ppm K (128 and 131% of control, respectively) and for females exposed to 30 ppm K (213% of control). These results indicate that repeated exposure to K 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. K 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.

Receptor binding studies done on animals receiving kepone for 90 days indicated significant decreases in dopamine binding in the corpous striatum and in QNB and GABA binding in the cerebellum; however, significant increases in protein were also observed, suggesting a lack of specificity. Serotonin and diazepam binding, or protein levels in the frontal cortex were not affected by exposure to kepone. Examination of neurotransmitter binding sites will take place in animals 30 days after cessation of exposure to

kepone. In vitro experiments on protein synthesis are also planned.

Blood taken from animals exposed to kepone for 90 days was also taken for neuroendocrine analysis. Results thus far indicate no significant alterations in circulating prolactin levels; other hormones will be measured.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90014-02 LBNT

PERIOD COVERED
October 1, 1979 to February 1, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Exposure to PBBs on Neurobehavioral Development of Mice

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:	J. A. Moore	Associate Director	RRP	NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	A. K. Agrawal	Visiting Fellow	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:
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)

Polybrominated biphenyls (PBBs) are environmentally prevalent agents with known toxic effects. To date, most toxicological experiments have dealt with adult organisms; the effects of PBBs on the developing organism have not been well studied. The purpose of this research is to determine the long-term consequences of exposing mice to PBBs during gestation and lactation.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Timed pregnant C57Bl/6 mice were dosed with 3 or 10 mg/kg FF-1, by gavage, every other day during gestation and until weaning at 21 days. Controls received corn oil vehicle. Offspring were given a battery of behavioral tests at 30, 90, and 120 days of age. FF-1 did not affect the body weights of the mothers or offspring, nor did it affect the number of pups or sex ratio of the litters. At 30 and 90 days of age, the startle response of the mice receiving 10 mg/kg was decreased, while mice receiving 3 or 10 mg/kg had decreased latencies in a test for negative geotaxis. At 30 days of age, males receiving 10 mg/kg had significantly increased emergence latencies, while at 90 days of age, the latencies of both males and females receiving either dose of FF-1 were significantly increased. Emergence latencies of all mice receiving 10 mg/kg of FF-1 were still increased at 120 days of age. Acquisition of an avoidance response was impaired at 60 days of age in all FF-1 exposed mice, but retention was not affected two weeks later. Exposure of mice to FF-1 during development appears to produce long-term alterations in the responsiveness of mice to some types of novel environmental conditions.

Receptor binding studies on animals exposed to PBBs during gestation have also been conducted. Six-month old mice exposed to FF-1 during development were found to have significantly reduced dopamine binding in the caudate nucleus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provides basic research into an animal model of environmental disease. These studies are of particular importance since they are designed to assess the potential of an environmental pollutant such as the PBBs to affect the long-term behavioral development of animals exposed during development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90018-02 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effect of Acrylamide on Dopamine Metabolism

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

PI:	A. K. Agrawal	Visiting Fellow	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
	L. L. Uphouse	Psychologist	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:
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)

Acrylamide has long been known as a neurotoxin, deleterious to the peripheral nervous system. More recently, this compound has been shown to have adverse effects on the morphology of the central nervous system. Since acrylamide poisoning is frequently associated with tremor, we are investigating the possibility that the locus of origin of some of these symptoms is the striatum. The metabolism of striatal dopamine is being studied from several aspects including assay of its levels, rates of breakdown, and receptor site density in acrylamide-treated rats.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Membranes are prepared from the striatum by lysis and centrifugation. A combination of freezing, thawing, and osmotic shock is used to destroy morphological structure so that high affinity uptake mechanisms are rendered inoperative. These membranes are then incubated in the presence of very low concentrations (around 10^{-9}M) of labeled ligands that generally are antagonists of the neurotransmitter whose binding site is being studied. The proportion of ligand binding is determined after filtration and isolation of the membranes from the incubation medium. Nonspecific binding is estimated by repeating such an incubation in the presence of a relatively high concentration (10^{-4}M) of unlabeled ligand or other antagonist. Such methods enable the calculation of dissociation constants, binding site density, reversibility, association velocity, and cooperative interactions between binding sites. Levels of dopamine, norephinephrine, and serotonin are measured by flurometric means.

MAJOR FINDINGS AND PROPOSED COURSE: In the adult male rat, either a single oral dose of acrylamide or ten doses over two weeks have the effect of enhancing binding of ^{3}H-spiroperidol to striatal membranes. This increase is due to an elevation in striatal dopamine receptor site density and also to an increased binding affinity of these receptors. This effect is rather specific in that for other transmitter receptors tested in various brain regions, are not significantly altered by acrylamide at doses which enhance dopamine binding.

Binding sites that are not greatly affected by acrylamide include receptors for GABA, norephinephrine, muscarinic acetylchloline, serotonin, glycine, and benzodiazepines. Effects on the dopamine receptor have been shown to be reversible within two weeks in the repeated dosing series of animals. Future work in this area will include:

(1) Determination of the time-course of this alteration of receptor number and finding out to what extent effects are directly dependent on protein synthesis.

(2) Measurement of striatal dopamine metabolites in treated and control animals by mass fragmentography. Levels of DOPAC can be taken as an index of the metabolic activity of dopamine.

(3) Determination of enkephalin levels in exposed rats by radio-immune assay. Enkephalin modulation of striatal dopamine circuitry is known to occur.

(4) Testing whether administration of FKF 525a (a microsomal oxidative system blocker by way of inhibition of cytochrome p-450), will exacerbate the effects of acrylamide. Such an inhibitory compound may retard the metabolic breakdown of acrylamide.

Other results from acrylamide-dosed animals have been obtained by neonatal or gestational exposure to acrylamide. In both these situations, the development of the striatal dopamine receptor appears to be retarded. This is, in contrast to the adult rat, where acrylamide has the opposite effect. The reversibility and specificity of these ontogenic phenomena will soon be studied. The possibility of a differential sensitivity of male and female pups is also under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: This work relates to the selectivity of acrylamide in its neurotoxic effects. It is relevant to regulation of acrylamide exposure that its mode of action is better understood. The relation between the biochemical and the behavioral changes induced by acrylamide may be clarified by this study. It may be that the tremor associated with acrylamide poisoning is related to abnormal dopamine metabolism, as is the case with Parkinson's Disease. This study may also provide a model for a new approach to the study of mechanism of neurotoxicity of a variety of other compounds.

The contrasting effects of acrylamide upon immature and adult animals illustrates the very different effects of toxic agents that are possible at various maturational stages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90019-01 LBNT

PERIOD COVERED
January 1, 1980 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Toxic Agents: Effects on Neuropeptide and Neuroendocrine Levels

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

PI:	J. S. Hong	Pharmacologist	LBNT	NIEHS
Other:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	C. Lamartiniere	Senior Staff Fellow	LOFT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	R. E. Squibb	Staff Fellow	LBNT	NIEHS

COOPERATING UNITS (if any)

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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:
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 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 steriod 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.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The endocrine or peptide content of serum or brain regions is measured by radio-immune assay using radio-iodinated antibodies that have been raised (generally in rabbits) against a range of compounds. Unknown samples are run together with a series of standards and the specificity of the antibodies is checked by electrophoretic and chromatographic means.

Care is taken not to stress animals during serum collection since anxiety is known to affect plasma endocrine levels.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary work has largely consisted of setting up the radio-immune assays and demonstrating their specificity and sensitivity. Serum prolactin levels have been shown to be considerably influenced by the means used to kill animals and have demonstrated the importance of minimizing stress.

The only toxicant that has so far yielded clear changes in hormones and neuropeptides is monosodium glutamate. Animals treated neonatally with this compound have been found to have severely reduced levels of β-endorphin within the hypothalamus while other neuropeptides such as neurophysin, met-enkephalin and substance P are unaltered in this region. Serum from male animals treated with monosodium glutamate at an early age had a significantly depressed content of testosterone which may account for the feminization observed in these animals.

Serum from rats exposed to acrylamide and kepone is currently being screened in a search for any abnormal hormonal concentrations that may result from these treatments.

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 radio-immune assays offers 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90020-01 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Abnormal Neurotransmitter Chemistry as a Test of Neurotoxicity

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
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	J. S. Hong	Pharmacologist	LBNT	NIEHS
	S. B. Por	Visiting Fellow	LBNT	NIEHS
	P. K. Seth	Visiting Scientist	LBNT	NIEHS
	R. B. Squibb	Staff Fellow	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	L. L. Uphouse	Psychologist	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:
5.1	3.5	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 α and β 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.

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.

Catecholamine levels are determined fluorimetrically. Membranes 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 RESULTS AND PROPOSED COURSE: Incubation conditions have been delineated for the following receptor sites. The labeled compound used to assay each receptor species is given in parentheses.

Dopamine	(spiroperidol)
Glycine	(strychnine)
GABA	(muscimol)
α-Noradrenergic	(dihydroergokryptine)
β-Noradrenergic	(dihydroalprenolol)
Serotonin	(serotonin)
Benzodiazepine	(diazepam)
Muscarinic cholinergic	(quinucidynyl benzilate)

To this list will soon be added:

Opiate	(naloxone)

The regional distribution, reversibility, specificity, and kinetic velocity at several temperatures have been determined for these interactions. The possible existence of endogenous inhibitory materials for these reactions has been surveyed and appropriate means have been employed to remove these. Other baseline parameters presently under survey include determination of several factors that influence receptor levels. These consist of developmental and circadian cycles and the effect of an enriched social environment. Preliminary findings suggest that receptor levels may fluctuate with time of day and may be modified by the nature of the environment in which a rat is maintained. The effect of denervation or prevention of normal innervation of brain regions is also being studied in the developing chick embryo.

A systematic survey of the effects of chemical treatment of animals upon a series of receptors is underway. 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 these 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. The dopamine receptor and levels of dopamine are depressed in the striatum of mice one year after gestation exposure to polychlorinated biphenyls (PCB's). These compounds are thus able to effect permanent changes in neuronal circuitry when given at an early stage of neural maturation.

The receptor systems have also been assayed in rats chronically exposed to dietary kepone. In this case there is no specific change in receptor density. Results are best explained by a general accretion of non-receptor containing membrane protein by the brain. Measurements of rats of protein synthesis in treated animals are planned in order to verify the suspected anabolic action of kepone.

Other chemicals are being used in dosing studies designed to examine the potential applicability of receptor binding phenomena as a test for neurotoxicity. These include tin and manganese.

The direct effects of toxicants upon receptor systems have been examined in vitro. Acrylamide at 10^{-5}M does not alter striatal dopamine binding and thus the reported effects of dosing must be attributed to an indirect effect on the dopaminergic system. This could either be due to effects on another parameter of the dopamine system (such as firing rates, or intensity of transmitter metabolism, or release) or to the existence of a metabolite of acrylamide that is the active agent. This latter possibility will be explored when more data is available about the distribution and metabolic fate of acrylamide within the rat (Z01 ES 90018-02 LBNT).

The in vitro 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 chloride.

Further results related to neurotransmitter binding are reported in Z01 ES 90003-03 LBNT and Z01 ES 90013-03 LBNT.

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 animal. 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.

Our work on the effects of heavy metal compounds on high affinity binding systems in vitro 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.

PUBLICATIONS

Damstra, T. E. and Bondy, S. C.: Neurochemcial Assay Systems for Assessing Toxicity. In Spencer, P. S. and Schaumburg, H. H.: Experimental and Clinical Neurotoxicology. The Williams and Wilkins Company, Baltimore, Maryland (In Press).

Damstra, T. E. and Bondy, S. C.: Neurochemical approaches to the detection of neurotoxicity. Target Organ Monographs, Raven Press, New York (In Press).

Bondy, S. C.: Rapid screening of neurotoxic agents by in vivo and in vitro means. Proc. 5th Ann. FDA Sci. Symp. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90021-01 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	C. L. Mitchell	Chief	LBNT	NIEHS
	A. K. Agrawal	Visiting 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	0.3	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)

Triethyl tin (TET) is an organo metal representative of a class of chemicals that produce vacuolization and splitting of myelin. To date, there has been a limited amount of work reported concerning the effects of TET on developing organisms. The following research concerns the effects of TET given during the postnatal phase of development of the rat. The purpose of the research is to: (1) attempt to identify dose related changes in behavioral functioning of rats when tested at different times after exposure (days 21-190 of age), (2) attempt to determine any long-term effects on neurotransmitter binding, and (3) attempt to assess the functional significance of any observed neurobehavioral and neurochemical effects using special psychopharmacological procedures.

-0040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Offspring of time-bred Fisher 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. Body weights were recorded at days 5, 7, 14, 21, 28, and 60 of age. At 21, 28, or 60 days of age, the rats were assessed in a battery of tests for developmental neurotoxicological effects. 60-day old rats were tested in a two-way shuttle box avoidance procedure, TET did not affect body weights at anytime during the study. Overall ANOVA indicated no significant effects of TET on fore- and hindlimb grip strength, negative geotaxis, or startle to an acoustic stimulus. Responsiveness to an air puff stimulus was decreased significantly by TET; the effect was evident at 21 and 60 days of age and occurred in both sexes. Spontaneous motor activity occurring in a 60-min period was increased by TET; the hyperactivity was evident in males at 21, 28, and 60 days and in females at 28 and 60 days. Latencies to emerge prior to the first shuttle box trial were significantly elevated in males that received 1.5 or 3 mg/kg TET and in females that received 3 mg/kg. Females receiving 3 mg/kg TET made significantly fewer avoidance responses; males were similarly affected, but the effect was not statistically significant. Activity during the intertrial interval was not affected by TET. These data indicate that a single postnatal injection of TET can produce long-term alterations in the sensorimotor capacities of rats and that the effect is independent of toxicant-induced changes in body weights.

Animals will also be assessed for neurobehavioral deficits and organ weights at 90 days of age.

Receptor binding studies have been initiated on animals that were sacrificed at 90 days of age. Significant decreases in dopamine but not QNB receptor binding in the corpous striatum have been observed in females receiving 3 mg/kg TET postnatally. Other brain regions (hippocampus, hypothalamus, cerebellum) are currently being assessed. The effects of TET on dopamine receptor binding appear to develop with age in that alterations in dopamine binding were not observed in animals sacrificed at 21 or 28 days of age.

The functional significance of the TET-induced changes in dopamine function is currently being assessed in several psychopharmacological models. These include apomorphine-induced stereotypy, apomorphine and areocoline induced disruption of operant behavior, and apomorphine/d-amphetamine-induced rotation in 6-hydroxydopamine-lesioned animals.

Studies on the postnatal effects of related chemicals (i.e., tetraethyl lead, hexachlorophene) are planned; experiments on the gestational effects of exposure to TET are also anticipated.

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 will provide some insight as to the types of behavioral tests that are appropriate for detecting developmental neurotoxicity and can lead to the elucidation of how long-term behavioral deficits can be caused by exposure to neurotoxicants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90022-01 LBNT

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Developmental Neurotoxicity of Acrylamide 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:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	C. L. Mitchell	Chief	LBNT	NIEHS
	A. K. Agrawal	Visiting 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	0.2	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)

Acrylamide is representative of a class of compounds known to produce "dying back" axonopathies. To date, there has been little work done on the potential neurobehavioral toxicity produced by exposure to these agents during development. The following research concerns the effects of acrylamide given during the pre- and postnatal period of development of the rat. The purpose of these experiments is to: (1) identify methods that are sensitive to any long-term neurobehavioral effects produced by developmental exposure to acrylamide, (2) attempt to establish dose-related changes in behavior, (3) attempt to determine long-term effects on neurotransmitter binding, (4) attempt to assess the functional significance of any observed neural deficits using special psychopharmacological procedures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: At 4 days of age, four male and four female Fisher-344 rat pups were randomly distributed to a foster mother. One pair from each litter was given 75 mg/kg AM (75 x 1) s.c. on day 5 and with distilled water (dH_2O) on days 7 and 9. A second pair was dosed with 25 mg/kg AM (25 x 3) on days 5, 7, and 9. A control pair was dosed with dH_2O on days 5, 7, and 9. Litters were weaned at 27 days of age. Startle responsiveness to an acoustic stimulus, spontaneous motor activity, fore- and hindlimb grip strength, negative geotaxis, and body weights were assessed at 2, 4, and 12 weeks of age. Acquisition of a two-way shuttle box avoidance response was tested at day 88 of age in males. The only behavioral measure affected by AM was the startle response and the effect was seen only in the males. At 15 and 30 days of age, the startle response was 43.4 and 67.6% of control for the 75 x 1 AM group, respectively, while the response was 29.1 and 55.4% of control for the 25 x 3 AM group, respectively. At 88 days of age, the startle response was significantly elevated for only the 75 x 1 AM group (153.9% of control). In the males, striatal DA receptor binding was decreased in the 75 x 1 AM group at 13 and 21 days of age (70 and 82% of control, respectively). DA binding of males in the 25 x 3 AM group and females receiving either dose of AM did not differ from controls. These data indicate that exposure to AM during the postnatal stages of development in the rat produces long-lasting behavioral and neurochemical changes and that there is a sex related sensitivity to AM exposure in the developing brain. DA receptor binding studies will also be done at 90 days of age.

Future experiments call for the assessment of receptor binding sites in the cerebellum, hippocampus, and hypothalamus of 21 and 90 day old animals having received acrylamide postnatally. Once the receptor binding work has been completed, special psychopharmacological tests will be used to determine the functional significance of the receptor binding changes.

A second series of major experiments has emphasized the gestational effects of acrylamide. In these studies, time-bred gravid rats were given 20 mg/kg of acrylamide or water vehicle on days 6-16 of gestation. The pups from each treatment group were either cross-fostered to a dam of the opposite treatment or kept with their natural mothers. Maternal body weights were not affected by the treatment; weights of the pups did not differ at any time up to 120 days of age. Behavioral deficits as assessed by a battery of tests (motor activity, fore- and hindlimb grip, negative geotaxis, startle, tail flick) were not observed at 28 or 60 days of age. More extensive tests (free operant activity, acquisition of a two-way shuttle box response) completed at 100 days of age also showed that acrylamide had no effects on behavior.

Neurochemical analyses of dopamine receptor binding of pups at 14 and 21 days of age showed a significant decrease in receptor binding at 14 days but not at 21 days of age. These data suggest that acrylamide delays the development of dopamine receptors. Additional experiments with acrylamide given gestationally will emphasize: (1) behavioral tests conducted during the preweaning

period, (2) different doses of acrylamide during gestation, and (3) different periods during gestation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developing organism is very sensitive to the presence of many types of environmental factors. It is highly relevant to our mission to develop tests capable of detecting alterations in neural functioning or the result of developmental exposure to environmentally prevalent chemicals. Acrylamide is representative of a class of compounds that produces dying back axonopathies and information concerning the mechanism by which it can produce developmental neurotoxicity might be applicable to a wide range of environmentally prevalent organic and volatile solvents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90023-01 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	O. A. Meyer	Visiting Scientist	LRDT	NIEHS
Other:	R. E. Squibb	Staff Fellow	LBNT	NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT	NIEHS
	V. Charles-Shannon	Interagency Personnel Agreement	LBNT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Reproductive and Developmental 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:
0.4	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)

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.

(Rev. 10-76)

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90024-01 LBNT

PERIOD COVERED
January 1, 1980 to September 30, 1980

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	Staff Fellow	LBNT	NIEHS
	J. A. Moore	Associate Director	RRP	NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT	NIEHS
	P. K. Seth	Visiting Scientist	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:
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)

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.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Female rats (3 or 4 to a cage) were fed 0, 0.2, 1, or 6 ppm of kepone (N=10 per group) via the diet for a period of 60 days, after which they were transferred to single cages containing a proven breeder male rat. Once breeding had been established, the females were housed individually. Kepone was made available to the pregnant animal. Exposure to kepone continued throughout gestation and during lactation until 12 days of age. The amount of food ingested and maternal body weights were taken weekly.

The body weights of the pups will be monitored for up to 180 days of age. Some animals will be assessed for neurobehavioral deficits at 2, 90, and 180 days of age (motor activity, grip strength, negative geotaxis, startle and tail flick). Shuttle box acquisition and responsiveness to d-amphetamine will also be measured. Animals will be sacrificed at 21, 90, and 190 days of age for neurochemical receptor binding studies and for neuroendocrinological analysis.

This study is currently in progress and data are not yet available.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90025-01 LBNT

PERIOD COVERED

October 1, 1979 to September 30, 1980

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:
1.6	0.8	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)

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.

6040
(Rev. 10-76)

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90026-01 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Catecholamine-Stimulated Adenylate Cyclase Inhibition by Alkyl Lead and Mercury Compounds

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

PI: 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:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The 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 cylase is usually inhibited at alkyl lead concentrations lower than those required for inhibition of basal adenylate cyclase activity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The adenylate cyclase assay is performed according to a slight modification of the method of Y. Salomon, C. Londos, and M. Rodbell, Anal. Biochem., 58, 541-548 (1974). The alkyl lead compounds were obtained from a commercial source, then purified so as to obtain compounds with relatively sharp melting points.

MAJOR FINDINGS AND PROPOSED COURSE: The dopamine-stimulated adenylate cyclase (DS-AC), in corpus striatum homogenates, is inhibited by mono-, di-, and triphenyl lead acetates under conditions where it is unaffected by inorganic lead acetate. The order of inhibitory effectiveness is such that the trialkyl compound is more effective than the dialkyl compound, which is more inhibitory than the monoalkyl lead salt; furthermore, the DS-AC is generally more sensitive than basal-adenylate cyclase (B-AC) to the alkyl lead compounds. Tri-n-butyl lead is more inhibitory to the DS-AC than is tri-n-propyl lead, which is more inhibitory than is triethyl lead acetate; again, DS-AC is inhibitied by these compounds more effectively than is B-AC.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: The DS-AC is currently thought to be one of the better understood transducing systems for the dopamine receptors present in the caudate nucleus. In a report by S. C. Bondy, it was observed that the dopamine receptor binding characteristics were altered at concentrations of tri-n-butyl lead of a magnitude similar to that which has been observed for inhibition of the DS-AC, thus, suggesting that such receptor modification could be of physiological and/or toxicological significance. Despite the prevalence of opinion concerning the probable role of the DS-AC as an important dopamine receptor transducing system, a number of recent reports have been concerned with efforts to test hypotheses that the DS-AC effects transduction of the consequences of interaction of various dopamine agonists and antagonists; in many instances involvement of the DS-AC has been found not to correlate positively with anticipated consequences of such agonist or antagonist interaction. Such lack of positive correlation might reflect the possibility that DS-AC does not represent a single functional or structural species, but is a mixture of molecular species. The results of the present project may lend support to the concept that the DS-AC represents multiple (possibly only two) molecular species and/or functional forms rather than a single molecular species.

An investigation of the mode of action of alkyl metal compounds as inhibitors of physiologically significant enzymes will help provide a better understanding of the enzymes under investigation as well as of those chemical and physical properties of agents which possess the capability of perturbing the enzymes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90027-01 LBNT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development of Biochemical Tests for Reflecting and Predicting Neurotoxicology

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

PI:	W. E. Wilson	Research Chemist	LBNT NIEHS
Others:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS
	J. S. Hong	Pharmacologist	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:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A variety of biochemcial 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.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Methods for most of the anticipated analyses will be adaptations of published techniques for analysis of proteins, nucleic acids, carbohydrates, lipids, amino acids, and a variety of enzymes. Several methods for analysis of neurotransmitter and/or neuromodulator compounds will be adapted to permit analysis using high performance liquid chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: Several biochemical parameters will be measured in tissues from various brain regions of rats exposed to acrylamide and/or carbon disulfide after the behavioral test design has been agreed to for those compounds.

The biochemical tests which are presently being considered are as follows: Tissue DNA will represent an indicator of cellular proliferation. Cell size will be reflected by the ratio of protein to DNA. The numbers of synapses will be reflected by tissue levels of putative transmitter metabolizing enzymes (i.e., dopamine β-hydroxylase, tyrosine hydorxylase, acetylcholinesterase, etc.) and/or by ganglioside concentrations. Physiologically relevant alterations in neurotransmitter metabolism will be reflected by profiles of transmitter compounds and their metabolites. The onset of and/or extent of myelination will be reflected by tissue levels of cholesterol, cholesterol esterase, and/or of 2',3'-cyclic nucleotide 3'-phosphohydrolase. Functional integrity of mitochondria will be reflected by tissue levels of citrate synthetase, Mg^{2+} ATPase, and/or succinic dehydrogenase. Mitochondrial density will be reflected by levels of monoamine oxidase. Plasma membranal functional ability will be reflected by levels of Na/K ATPase and/or transmitter- or modulator-sensitive adenylate cyclase. Endoplasmic reticulum functional ability will be reflected by levels of NADPH-cytochrome reductase. Lysosomal degeneration will be reflected by tissue levels of β-glucuronidase and/or β-galactosidase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: A wide spectrum of biochemical alterations can be expected to occur in the central and peripheral nervous systems as consequences of exposure of animals to various toxic agents. It may be possible to develop a profile of responsiveness of various biochemical parameters susceptible to modification by each of several toxic agents. Out of such an endeavor may evolve a method for recognizing those biochemical parameters whose alteration might permit prediction of likely behavior modification (or other toxicological response to the agent under study). Such an effort may also lead to the development of a screen suitable for anticipating toxic responsiveness to compounds which are structurally related to the toxic agent(s) under investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 90028-01 LBNT

PERIOD COVERED

May 1, 1980 through September 30, 1980

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	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	0.2	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)

2,4-Dichlorophenoxyacetic acid (2,4-D) is a widely used herbicide and a component of Agent Orange. There have been several reports of delayed neuropathy following exposure to this agent. The purpose of this research is to provide a profile of the neurobehavioral toxicity in rats with special emphasis on its effects on motor performance.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fifty male and fifty female, 90-day old Fischer 344 rats ere administered 0, 100, 200, 400, or 800 mg/kg 2,4-D orally in corn oil in a volume of 0.2 ml/100 g body weight. Periodic visual assessment of neurotoxicity was made according to methods described by Irwin (Psychopharmacologia 13: 222-257, 1968). In addition, quantitative measurements using a neurobehavioral test battery developed in this laboratory were made. Assessments were made for a six-hour period following dosing and 1, 2, 3, 5, 7, 14, and 28 days postdosing. The number of lethalities were also noted.

In a subsequent experiment, similar observations will be made in animals dosed over a two-week period.

MAJOR FINDINGS AND PROPOSED COURSE: The two most prominent signs observed have been labored breathing, hindlimb weakness, and ataxia. Deaths occurred over a period of four days in the 400 and 800 mg/kg groups of males. Females appear more resistant to the effects of 2,4-D than males. Future studies will concern the effects of more prolonged exposure with lower doses.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 2,4-D is a widely used herbicide. The consequences of exposure to 2,4-D on neurobehavioral functioning have not been studied systematically.

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 Fisher-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 for acrylamide and tetraethyl tin. Studies on kepone and arsenic are underway. The neurotoxic profile generated by the contractor for acrylamide is similar to that observed in-house (i.e., degenerative muscular weakness indicative of peripheral neuropathy). The only effect noted for tin after 15 weeks was a change in startle response. The data, thus far, suggest that the high dose used in the 15-week study needs to be higher than that derived from the formula (i.e., 2/3 of LD20). Subsequent experiments will employ the LD20, 2/3, and 1/3 of the LD20 in the 15-week study.

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 bio-

medical 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 the goals within the shortest possible time, several mechanisms are used. These include:

1. Development of an intramural research program based on inhouse research by staff scientists and support of work at other institutions in the collaborative program with staff scientists as project officers.

2. Development of better mechanisms for information exchange. These include: (a) support of the Environmental Mutagen Information Center (EMIC) and (b) serving as associate editor or members of the editorial board of journals which publish research in the area of environmental mutagenesis.

3. Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at: (a) the predoctoral level and (b) the postdoctoral level.

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. Malling and Ansari are utilizing the various hemoglobins of the inbred strains of mice which differ in as many as thirteen amino acids. Strong monospecific antibodies have been produced to one of the types. The mutant cell can be detected by using the monospecific antibodies which are flagged with a fluorescent molecule. Sperm of mammalian species contain a specific lactic dehydrogenase-X (LDH-X). In collaboration with Mr. Burkhart this enzyme has been localized as an essential part of the tail. Interspecies antigenic differences are being utilized for detection of mutations. Drs. Ansari and Malling have developed the technique for detection of mouse sperm which react with monospecific antibody 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. Dr. Malling is using histochemical methods to detect heat- and urea-resistant enzymes in individual sperm. 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. To at least get a handle on this problem, Mr. Burkhart and Dr. Malling have studied the genetics and the ultrastructural mechanism of one particular sperm mutation. Induction of translocations from a mutagenic environment could impose a serious health hazard for the human population. The present tests for translocations are laborious and expensive. Mr. Burkhart and Dr. Malling are developing a new technique to detect translocations directly in sperm.

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.

COLLABORATIVE RESEARCH PROGRAM

In the Collaborative Research Program most of the contracts fall naturally within two areas: (1) development of systems for monitoring of the human population for mutations and (2) dissemination of mutagenesis information. The contracts will be described following this outline.

1. Mutation monitoring systems for the human population - In a contract at the University of Washington, an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing mutant hemoglobin rather than normal hemoglobin. Specific antisera are being developed to detect red blood cells, each of which carry one of ten different hemoglobins. By conjugating the antiserum with different colored fluorescent dye, mutant RBC's will fluoresce.

2. Automatic detection of variant cells - Under an interagency agreement with the Lawrence Livermore Laboratory, attempts are made to develop methods for detection of rare variant cells among 10^6 or 10^7 normal cells. This requires stains of the red blood cell with the fluorescent antibodies in suspension. The technical problems in the staining procedure have been partially solved. The present sorter is not of sufficient sophistication to descriminate between normal and the rare cells at frequencies less than 10^{-4}. A new type of sorter (slit-scan) is therefore being developed and is available for this research. In the future the work will use this new sorter.

3. Collection and dissemination of mutagenesis literature - During the past year we have continued to support the Environmental Mutagen Information Center (EMIC) by interagency agreement at the Oak Ridge National Laboratory. This Center has over 30,000 bibliographic entires in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

INFORMATION EXCHANGE

Journals - Staff members serve as associate editors, assistant editors, or members of editorial boards of Mutation Research, Environmental Health Perspectives, The Journal of Toxicology, and Reviews in Genetic Toxicology. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

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 included three visiting fellows from two foreign countries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60019-07 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Use of Male Germinal Tissue in the Detection of Mutational Events

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

PI:	H. V. Malling	Laboratory Chief	LBG	NIEHS
Other:	M. Snell	Bio. Lab. Tech.	LBG	NIEHS

COOPERATING UNITS (if any)
Environmental Biology & 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:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The aim of this project is to develop systems for measuring the frequency of point mutations in male germinal tissue. These systems would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa. At present enzyme histochemical methods are being developed to detect point mutations in male mice. The histochemical staining technique has been quantitized for α-glycerophosphate dehydrogenase and succinyl dehydrogenase. Techniques have been developed for selective staining of sperm containing thermo-resistant or urea-resistant α-glycerophosphate dehydrogenase and for sperm containing Malonic acid resistant succinyl dehydrogenase. Mutation frequencies for urea-resistant sperm have been determined after treatment of male mice with procarbazine.

PROJECT DESCRIPTION

METHODS EMPLOYED: Some enzymes in sperm can be detected readily by histochemical methods. The enzymes under study in this laboratory are α-glycerophosphate dehydrogenase and succinate dehydrogenase.

The principle of these studies is based on sperm which contain enzymes resistant to urea, thermo-denaturation, and resistance to malonic acid which is the competitive inhibitor of succinyl dehydrogenase. The mutants are detected as having positive enzyme activity under the selected conditions. These mutations will be estimated by counting stained and unstained cells with the aid of a Zeiss Axiomat microscope connected to a PDP-12 computer and a teleprinter output. Information also will be collected on magnetic tape for processing in the Institute's PDP-11 computer.

MAJOR FINDINGS AND PROPOSED COURSE: Thus far we have developed histochemical methods for α-glycerophosphate dehydrogenase (α-GPD) and succinic dehydrogenase. The dehydrogenases were localized by using nitro blue tetrazolium. The accumulation of stain in the single midpiece was measured directly by use of a Zeiss Axiomat microscope and a photomultiplier tube. After heating of sperm from DBA/2J mice to 63°C for 60 minutes most sperm do not stain at all; however, a few sperm were stained strongly. It is possible that these sperm could be mutants containing thermo-resistant enzymes. Similar techniques have been developed for detection of sperm containing α-glycerophosphate dehydrogenase resistant to urea denaturation. Techniques have also been developed for the detection of sperm containing succinyl dehydrogenase that are resistant to malonic acid. The genetic alterations which will result in a sperm with an enzyme resistant to adverse conditions are likely to be very specific. The proposed course of this investigation is to continue to expand the number of selection criteria employed for funding the mutants. These will include screening for derepression of enzymes which only occur in somatic cell etc.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system is important, because it would permit the detection of mutational events within a single individual that could be used to monitor the human population for mutations in the male germinal cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60021-08 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Gene Mutation Indicators 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

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Electrophoretic methods, spectrophotometrically determined enzyme characteristics, morphometric features, and metabolite profiles are being investigated for their potential to indicate newly occurring mutational events in mice. The purpose of the work is to seek more effective means to detect germinal mutations while also providing greater understanding of individual mutational effects and of increasing mutation rates generally, on the mammalian organism. The work is directly related with human genetic health risks caused by environmental mutagens. Experimental mutagens which have been used include methylmethane sulfonate, procarbazine, and ethylnitrosourea. A considerable number of spontaneous and induced mutants have been identified. These are being characterized by a variety of criteria.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this work are the development of methods to detect germinal mutations in mice and to provide understanding of the impact of mutations in the context of genetic risk. Characteristics and methods which are being explored are enzyme properties as reflected by starch gel electrophoresis and isoelectric focusing, enzyme activities determined by centrifugal fast analyzer, metabolic profiles analyzed by chromatographic techniques, and physical dimensions of the skeleton measured with the aid of an X-Y digitizer/computer system. 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 phosphoglucomutase, malic enzyme, isocitrate dehydrogenase, hemoglobin, and others have been found. After genetic confirmation, mutant gene products are characterized by various criteria. Later, the impact of the mutations on the life functions of the animals will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance to human health of germinal mutations and increased germinal mutation rates is not well understood. Two needs are critical if the problem is to be probed effectively, (1) an efficient and economical series of methods capable of detecting various classes of mutational events in material sampled directly from humans and (2) a whole animal model system which can be manipulated experimentally. The mouse, the animal with which this project is directly concerned, provides a convenient source of material for methods development; and because of the many established homologies with man, some of the methods used for mice, plus some of the mutations and effects observed for mice, are readily relatable to humans. As methods are provided with the mouse, model system capability is also generated. With this it should be possible to test directly the genetic effects of specific environmental mutagens and explore generally the fundamental relationships concerning mutations and their impact on fitness and health.

PUBLICATIONS

Roberts, G. T., Johnson, F. M., Malling, H. V., and Sharma, R. K.: Action of N-isopropyl-α-(2-methylhydrazino)-p-toluamide hydrochloride (procarbazine hydrochloride) in germ tissue of mice: Dominant lethal effects. Arch. Toxicol. 41: 287-294, 1979.

Sharma, R. K., Roberts, G. T., Johnson, F. M., and Malling, H. V.: Translocation and sperm abnormality assays in mouse spermatogonia treated with procarbazine. Mutation Res. 67: 385-388, 1979.

Lee, C.-Y., Chasalow, F., Lee, S.-H., and Johnson, F. M.: A null mutation of cytoplasmic malic enzyme in mice. J. Mol. Cell Biochem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65002-03 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

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:
0.3	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 goal of this project is to develop and test systems that could be used to study mutagenesis in mammals using 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.

FORM 2-6040
(Rev. 10-76)

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.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65007-03 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

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:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project was undertaken to study mutagenesis using monospecific antibodies against the sperm specific enzyme, lactate dehydrogenase-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 mitomycin C and procarbazine treated mice.

PHS-6040
(Rev. 10-76)

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 hours 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 AND PROPOSED COURSE: In the last report (Z01 ES 65007-02 LBG) scoring of presumptive mutant mouse sperm from control and procarbazine-treated mice was reported. Subsequent to that report, a blind screening of slides was carried out in which the slides prepared from control and different groups of procarbazine-treated mice were coded by a person not associated with these studies. A linear dose vs. response relationship, like the one reported earlier, was obtained in the blind screening. In another experiment, procarbazine was injected to the mice in multiple injections (three injections) instead of just one injection. The presumptive mutation frequency of 2.6×10^{-6} for 300 mg/kg total dose was found to lie between the values obtained for 200 and 400 mg/kg single injection doses. Also, the frequency of 6.4×10^{-6} for 600 mg/kg cummulative dose lay close to the value for 600 mg/kg single dose group. These results indicated that procarbazine had a cummulative dose effect.

The second drug tested in this system is mitomycin C. Treatment of mice with mitomycin C from 2 to 7 mg/kg dose also caused increase in the presumptive mutation frequency up to 11.8×10^{-6}.

Mutation frequency. The monospecific antibody used in these studies interacts with rat LDH-X and not with mouse LDH-X. However, if the mouse LDH-X undergoes mutation such that an amino acid normally present in the

mouse LDH-X is replaced by one present in rat LDH-X at a position where the rat and mouse enzymes differ immunologically, this antibody would interact with the mutant molecule. Thus, normal mouse sperm would not be stained but the mutant mouse sperm will be fluorescent.

We found 72 such mutant sperm out of 167 x 10^6 sperm from normal DBA/2 mice giving a spontaneous mutation frequency of 0.43 x 10^{-6}. Mice treated with increasing dosage of procarbazine showed an upward trend in the mutation frequency; the procarbazine dose (mg/kg) and corresponding mutation frequency are as follows: 200, 1.94 x 10^{-6}; 400, 3.66 x 10^{-6}; 600, 5.47 x 10^{-6} and 800, 9.17 x 10^{-6}. These data represent an almost linear dose vs. response relationship.

Proposed course: (1) Automation. Scanning the slides for locating the mutant sperm is a very tiring and time-consuming task. A computerized automatic scanning system will be developed. (2) More mutagens. Dose vs. response relationship has been obtained for procarbazine. Similar experiments will be done using other known mutagens, e.g., ENU and TEM. After it has been demonstrated that the system works with known mutagens, it will be tried with chemicals that are suspected mutagens or carcinogens. (3) Screening systems. It is hoped that after several known and unknown mutagens have been successfully tested with the sperm system, the method could be accepted as a screening system for mutagenic activity of chemicals. This will be an *in vivo* mammalian screening system. (4) Monitoring system. Monospecific antibodies will be developed that would react only with monkey LDH-X and not with human LDH-X. Such antibodies will be used to study mutation from human to monkey type LDH-X. The utility of this system would be for monitoring human population for genetic changes.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65009-02 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Detection of Translocation Carriers by DNA Measurement 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
	T. G. Riddle	Biomedical Engineer	Contract Support	

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:
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)

The objective of this project is to develop methods to detect translocation carriers in populations of mice treated with potential mutagens by evaluating the DNA distributions of sperm. 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 and those carrying known translocations. Work has begun to examine the feasibility of automatic systems of measurement and analysis.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Meiosis in male translocation carriers results in sperm that have normal and abnormal amounts of DNA. Fluorescent labels can be used to quantitate DNA by measuring the relative intensity of emitted light upon excitation of the fluorochrome. In principle, the variance of the distribution describing DNA fluorescence of sperm populations will be greater in individuals with translocations. Fluorescence will be measured with the Axiomat microscope. Data on single cells and populations will be collected and analyzed by the PDP-11 computer.

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 significantly 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 F_1 males derived from parents treated with compounds known to induce chromosome abnormalities will be evaluated. Data will be correlated to other tests used to detect translocations such as the fertility test and cytogenetic analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system would significantly reduce the time cost and animals needed to assess the potential of compounds to induce chromosomal damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65011-01 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Derepression of LDH-X in Mouse Hepatocytes

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

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	0.1	0.5

CHECK APPROPRIATE BOX(ES)

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☐ (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. 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 frequency of hepatocytes reacting with anti LDH-X has been measured in male and female DBA/2J mice.

PHS-6040
(Rev. 10-76)

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. This suggests there is a measurable spontaneous frequency of abnormal derepression of specific gene products. Future investigations will establish a normal range for frequency of LDH-X expression in hepatocytes of mice and determine the effects of hepatoctomy and exposure to known mutagenic and carcinogenic agents. 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65012-01 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Plaque Forming Cell Assay for Mouse Immunoglobulin Allotypes

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:
0.7	0.6	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 goal of this project was to develop reverse plaque assay for mouse immunoglobulin allotypes. Myeloma proteins were used to raise antibodies in various species of mice and rabbits. Possibilities whether these antibodies could be used to develop reverse plaque assay for detection of mutation in mouse immunoglobulins were explored.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Purification of mouse immunoglobulin allotypes: Tumors secreting MOPC 173, MOPC 167, MOPC 382, MOPC 603, HOPC 1 and EPC 109 myeloma proteins were grown in intraperitoneal cavity of mice. The myeloma proteins from the ascitic fluids thus obtained were purified by affinity chromatography on protein A-Sepharose column.

2. Preparation of antibodies: For producing antibodies against mouse immunoglobulin allotypes, the myeloma proteins were injected into various strains of mice and rabbits. Each antiserum thus obtained was absorbed twice on sheep red blood cells (SRBC) before use. Gamma globulins from the sera were precipitated by sodium sulfate. The precipitate was dissolved and dialyzed against normal saline. Balb/c anti-C57IgG2a and rabbit anti-MOPE 352 were made monospecific by repeated absorption on DBA/2 immunoabsorbent.

3. Reverse plaque assay: The method of reverse plaque assay involves the lysis of antibody-coupled sheep red blood cells (SRBC) in the presence of corresponding antigen and complement. In the case of mouse immunoglobulin allotype system, a polyvalent antibody against mouse Ig is coupled to SRBC. These indicator cells are mixed with mouse lymphocytes, the anti-allotype antibody, and complement. This mixture is poured into chambers, prepared with the help of two microscope slides and double coated type, which are sealed by wax. After an incubation period, a clear circular zone is formed around any lymphocyte which is secreting the same Ig allotype against which the anti-allotype antibody was raised, because the antigen (Ig allotype) reacts with the antibody and this complex activates the complement which leads to the lysis of SRBC in the immediate vicinity of the lymphocyte secreting the antigen. Instead of coupling the polyvalent antibody, the anti-allotype antibody or Staphylococcus protein A — which interacts with immunoglobulins — can also be coupled to SRBC and these cells may be used as indicator cells. All these alternatives were tried. A typical reaction mixture consisted of 90 µl of HBSS, 20 µl of indicator cells (SRBC coupled with goat anti-mouse γG or Protein A), 25 µl of spleen cells from the test mouse (C57BL/6 or DBA/2), 20 µl of the monospecific antibody (rabbit anti-MOPC 352) and 20 µl of appropriately diluted guinea pig complement.

MAJOR FINDINGS AND PROPOSED COURSE: Chromium chloride is the reagent which is generally used for coupling of proteins to SRBC. We found that most of the antibodies we used, such as rabbit anti-MOPC 352, rabbit anti-MOPC 173, Balb/c anti-C57BL/6 IgG2a etc., lose their activity after they are coupled to SRBC by chromium chloride. However, goat anti-mouse γG was found to retain its activity after coupling to SRBC. Therefore, instead of coupling monospecific anti-allotype antibodies directly to SRBC, goat anti-mouse γG or Protein A were coupled to SRBC and these cells were used as indicator cells. Monospecific antibodies were used as developers of the plaques. Homologous antibodies against mouse Ig allotypes producpd in various strains of mice did not form plaques, probably because they do not fix complement. However, antibodies produced in rabbits were effective in forming the plaques. In order to test the worth of this system, rabbit anti-MOPC 352 was made monospecific. This antibody was found to form plaques with only C57BL/6 spleen

cells and not with spleen cells from DBA/2 mouse. Since the antibody detects only allotype IgG2b.9,16,22,33,34 producing lymphocytes, it was necessary to determine the total number of Ig producing lymphocytes and the number of lymphocytes producing different subclasses of Ig. Only 1000 plaque forming cells were found per million of C57BL/6 lymphocytes out of which 77 were IgG2b producing cells. In the case of DBA/2 mouse, 1228 plaque-forming cells per million of lymphocytes were observed out of which only 78 were IgG2b producing. Maximum number of lymphocytes which can be taken per plaque chamber without affecting the number and morphology of the plaques was found to be about 6 million. This means that only about 470 IgG2b producing lymphocytes can be taken in each chamber. Therefore, for screening 10^6 IgG2b producing lymphocytes, 2128 chambers will have to be prepared. This will be quite cumbersome.

It is proposed to develop a system, employing the technique of immunofluorescence that can be used to detect mutation in lymphocyte cell surface genetic markers. To this end, the monospecific antibodies will be labeled with a fluorescent compound, such as fluorescein isothiocyanate or rhodamine isothiocyanate and will be used to detect the corresponding immunoglobulin allotypes on the surface of lymphocytes from a test mouse.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The major goal of the Laboratory of Biochemical Genetics is to develop systems that can be used to study mutagenesis in mammals using readily available samples. If the above-mentioned approach succeeds, it will form a strong basis for development of such a system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER: (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65013-01 LBG

PERIOD COVERED
October 1, 1979, through September 30, 1980

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:
0.7	0.6	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 was 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 this method for detection of mutation in mouse hemoglobins is being worked out.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Preparation of antibodies. Antibodies against hemoglobins of two strains of mice, C57BL/6 and DBA/2, were produced in horse, rabbit, and various other strains of mice. Each antiserum was absorbed twice on sheep red blood cells. Gamma globulin fraction was isolated by precipitation with 18% sodium sulfate. The precipitate was dissolved and dialyzed against saline.

2. Plaque assay. The method of plaque assay involves the lysis of antibody-coupled sheep red blood cells (SRBC) in the presence of corresponding antigen and complement. The idea of hemoglobin plaque assay is novel. It can be performed by coupling of monospecific antibody against a particular mouse hemoglobin variant to SRBC. These indicator cells are mixed with the mouse red blood cells carrying that hemoglobin variant, complement, a developing antiserum and antibody against mouse rbc ghost. The mixture is poured into the plaque chambers which are sealed. The anti-ghost lyses the mouse rbc releasing the hemoglobin in the medium. This released hemoglobin then complexes with the monospecific antibody present on the SRBC surrounding the mouse rbc and activates the complement and this results in the lysis of the indicator cells forming a plaque. These are counted by the naked eye.

MAJOR FINDINGS AND PROPOSED COURSE: Anti-mouse hemoglobin antibodies produced in horse, rabbit, and various strains of mice were found to loose their activity when coupled to SRBC by chemical methods. Therefore, an indirect method for the coupling of the antibodies to SRBC was employed. First, protein A was coupled to SRBC by chromium chloride method. These coupled cells were washed four times with saline and then incubated with γG fraction of anti-hemoglobin antibody (LP anti-C57BL/6 Hb) for 10 min at room temperature with constant shaking. The cells were then washed twice with normal saline and used as indicator cells. For the assay, total volume of the reaction mixture was 170 μl of 5% final concentration of fetal calf serum and it contained the following: 25 μl of the indicator cells, 25 μl of mouse rbc from the test animal, 30 μl of 1:9 diluted rabbit anti-C57BL/6 hemoglobin, γG globulin fraction, and 20 μl of 1:3 diluted guinea pig complement. The volume was made up with Hank's balanced salt solution. Plaques begin to appear after about 2 hrs when the chamber is incubated at room temperature and they are fully developed within 3-4 hrs when they can be counted with an unaided eye. It was found that for the lysis of mouse rbc, addition of an antibody against mouse rbc ghost is not necessary because the developing antibody, rabbit anti-C57BL/6 hemoglobin, contains sufficient anti-ghost to lyse the mouse rbc under test. The initial results show that LP anti-C57BL/6 hemoglobin forms plaques with rbc from both C57BL/6 and DBA/2 mice. The antibody has to be made monospecific by absorption on immunoabsorbent such that it gives plaques only with C57BL/6 rbc and not with DBA/2 rbc. Attempts are now being made in this direction.

Attempts will be made to make LP anti-C57 Hb antibody monospecific such that it forms plaques with C57BL/6 rbc or rbc from any other mouse which contains s hemoglobin and not with DBA/2 rbc which carry d hemoglobin. This monospecific antibody will be used to study mutation frequency — spontaneous as well as

induced after the treatment with some known mutagens. After testing the work of the system with known mutagens, other potential mutagenic substances can also be tested by studying the dose-response relationship.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: When fully developed, the method could be used to identify chemicals 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 programs.

INFORMATION CENTER COMPLEX/INFORMATION DIVISION
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE 37830
(Interagency Agreement Between NIEHS and ORNL)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTES: National Cancer Institute; Energy Research and Development Administration

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information.

MAJOR FINDINGS AND PROPOSED COURSE: EMIC's data file at the end of March 1980 contained 30,017 bibliographic entries. Of these, approximately 28,014 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number(s). The Center processes requests for information at a rate of 8 per day. These requests come primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received. The EMIC data bank can now be screened by Tox-Line. EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objectives as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information for the scientific, educational, and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with the time state-of-the-art reports on subjects of current scientific interests.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators, and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (2437). EMIC has helped several national commissions in collecting and extracting the

data from the literature. For the Institute, and especially the Laboratory of Biochemical Genetics, EMIC saves more than one man-year of time for the personnel in the Laboratory by supplying their information needs.

PUBLICATIONS

Wassom, J. S., Shelby, M. D., Von Halle, E. S., Beauchamp, R. O., Jr., Owens, E. T., Miller, I. R., Barnard, W. J., and Adams, J. O. Chemical Mutagenesis: A Survey of the 1975/1976 Literature. Oak Ridge National Laboratory Publication No. ORNL/EMIC-10. Oak Ridge National Laboratory, Oak Ridge, TN, pg. 502 (1979).

Brown, M. L., Wassom, J. S., Malling, H. V., Shelby, M. D., and Von Halle, E. S. Non-mammalian assay systems used in the evaluation of selected chemical compounds for mutagenic activity/A literature summary. J. Natl. Cancer Inst. 62: 841-871, 1979.

Larsen, K. H. Indexed collection of the literature on aneuploidy. Environmental Health Perspectives 31: 167, 1979.

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON
(NIH-NIEHS-74-2151)

TITLE: Detection of Point Mutations in Somatic Cells

CONTRACTOR'S PROJECT DIRECTOR: G. Stamatoyannopoulos, M.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED: June 1, 1979

CURRENT ANNUAL LEVEL: $195,000

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many different types of hemoglobin mutations exist in the human population. Some of these are point mutations in the α- and β-chain. Since such mutations occur in the germinal tissue, it is reasonable to assume that they also occur in the stem cells for the red blood cells (RBC). This type of mutation should result in an RBC which would contain an aberrant hemoglobin. By using a monospecific antibody to a number of aberrant hemoglobin types, such cells should be detected in samples from normal individuals after reaction of the antibodies with various fluorescent dyes. Antibodies are being produced against Hb S, Hb C, HbO Arab, Hb E, HbQ India, Hb Ottawa, Hb Hasharon, Hb Cranston, Hb Wayne, and Hb Constant Spring. Antibodies which are produced in animals vary in quality. In contrast monoclonial antibodies produced by hybridomas are uniform. Attempts are being made to produce monoclonial antibodies from hybridomas to Hbb S which do not react with Hbb A.

MAJOR FINDINGS AND PROPOSED COURSE: Most of the monospecific antibodies have been produced against variants of hemoglobins due to base-pair substitutions. Hemoglobin Wayne and hemoglobin Cranston are frameshift mutants. Purification of monospecific antibodies against these hemoglobin variants was difficult. Sufficient amounts of antibodies now exist to carry out the first attempt to detect red blood cells containing Hb Wayne and Hb Cranston.

For the future development of this research, it will be necessary to produce monoclonial antibodies from hybridomas. The advantages by using monoclonial antibodies are uniform affinity and high specificity. Several fusions have been made between spleen and lymphoid cells from Balb/c-2 mice and myoloma cells. The fusion has been successful and many IgM producing clones have been isolated. A clone producing IgG has not yet been isolated. The fusion will

continue using Balb/c, DBA/1J and SLJ mice. In addition to the animals to be immunized with Hb S, groups of mice will be immunized with Hb Wayne, Hb Cranston, and Hb C.

In order to supply Lawrence Livermore Laboratory with monospecific fluorescent antibodies, the production of anti-Hb S and anti-Hb C has continued. These antibodies will be used in the genetic proof of the mutant nature of the variant cells. The mutation frequency of RBC's which react with Hb S in a normal human is 10^{-7}. Hb C cells occur with a similar frequency. The mutation from Hb A to Hb S or Hb C requires one base-pair substitution each. A mutation from Hb S to Hb C or visa versa requires two base-pair substitutions. In practicality the frequency of Hb C cells in an Hb S individual should be 10^{-14} if these cells originate through mutations.

Antibodies to an expanding list of hemoglobin variants will be produced and used to investigate somatic cell mutation rates with respect to age and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect mutations in the genome of readily accessible somatic cells would allow estimation of the mutation rate in the human population. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PUBLICATIONS

Stamatoyannopoulos, G.: Possibilities for demonstrating point mutations in somatic cells, as illustrated by studies of mutant hemoglobins. In, Genetic Damage Caused by Environmental Agents, edited by K. Berg, Academic Press, New York, Pg. 49-62, 1979.

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system using Hb mutants. IV. Successful detection of red cells containing a human frameshift mutant Hb Wayne and Hb Cranston using monospecific fluorescent antibodies. Am. J. Human Gen., in press.

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LAWRENCE LIVERMORE LABORATORY - LIVERMORE, CALIFORNIA 94550
(Interagency Agreement Between NIEHS and Lawrence Livermore)

TITLE: Cytophysical Studies in Mutagenesis

CONTRACTOR'S PROJECT DIRECTOR: Mortimer L. Mendelsohn, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

COLLABORATING INSTITUTES: Department of Energy

DATE CONTRACT INITIATED: September 23, 1977

CURRENT ANNUAL LEVEL: $210,000

PROJECT DESCRIPTION

OBJECTIVES: Development of system for monitoring of the human population for mutations in vivo in single cells depends on the detection of a rare event in a single cell which is most effectively done by using flow systems. The scope of this contract is to develop such systems for red blood cells and sperm.

MAJOR FINDINGS AND PROPOSED COURSE: At the present time it takes about one person-month to manually estimate the spontaneous mutation rate from Hb A to Hb S in a human. In order for this system to be practical the counting must be automized. This could be done through application of the flow system. A technique has been developed for labeling of red blood cells with fluorescent antibodies. The important step was to cross-link hemoglobin on the cytoplasmic side of the membrane. The flow system can now detect rare fluorescent ghosts in the midst of a large excess of unstained ghosts with total throughput rates of $\geq 10^6$ per second. Attempts will be made to increase the intensity of the AS cells and to improve the antibody preparations to eliminate precipitated protein as a source of false-positive signals. One likely solution to this problem is slit-scan flow cytometry. In this method fluorescence is detected as objects flowing through a narrow ribbon of laser illumination. Specks and uniformly fluorescent ghosts should be readily discriminated by the difference in the duration of their fluorescent signals.

The technique for staining of the red blood cells in suspension with fluorescent markers has successfully been expanded to the following monospecific antibodies: anti-C, anti-Cranston, and anti-Wayne.

SIGNIFICANCE TO THE BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic or carcinogenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. Nevertheless we do not have available today a monitoring system for the human population. Through collaboration of our inhouse research, University of Washington, and this contract, we hope to develop such systems.

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. An exposure chamber for single animals, which enables the monitoring of physiological parameters during exposure, has also been developed.

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 if pulsed wave microwave radiation at 2450 MHz produced a different effect than 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 significant 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 were 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 peritioneal mast cells were exposed to 2450 MHz microwave radiation at specific absorption rates (SAR) of 8.5 and 42.5 mW/gl 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.

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.

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 + 22.3 mW/g) did not produce any adverse maternal or embryofetal effects. At exposure to 30 mW/cm^2 (SAR = 32 mW/g) during days 1-6 a significant decrease in implantation sites per litter and average fetal weight was observed. Exposure to 30 mW/cm^2 during days 6-15 resulted in a slight increase in the number of malformed fetuses (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 cavity 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.

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-deoxyglucose 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 which 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 the additional hearing loss which occurs; study those specific non-auditory systems (endocrine, immunologic, physiologic, pharmacologic, teratogenic, cardiovascular) which may be affected by chronic noise exposure; identify by appropriate epidemiological methods those factors which are related to hearing loss and the non-auditory effects of noise.

Areas under current investigation include: correlation between the cochlear potentials and ion movements within the cochlea and their alteration by exposure to noise; 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 and noise effects upon the fetus.

Considerable progress has been made towards 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. Measurement of electrochemical profile for potassium in the cochlear hair cells under normal and pathological conditions is now being carried out, since our results demonstrate the critical role of potassium ions on generation of the receptor potential. The combined effect of noise and ototoxic agents will be examined in future studies.

A prototype impact noise generator has been developed and its acute effect on guinea pig cochlear function compared with that of continuous noise exposure of equal energy. Most damage risk criteria are based on the assumption noises of equal energy produce similar cochlear damage. Our studies show that in short periods of exposure impact noise produces greater suppression of the cochlear microphonics while continuous broad band noise of equal energy gives rise to a greater suppression of the action potential. Effects of exposure to impact or continuous noise of long duration will be compared using a pneumatic drium noise generator to assess whether a pneumatically driven generator to assess whether the results found in short term experiments are duplicated in chronic experiments.

Studies on the coding of complex signals in the auditory system have continued. In this work speech-like inputs consisting of pseudorandom noise which was been amplitude modulated by sinusoidal signals are recorded in the form of a cycle histogram. The cross-properties between output and known input, which represent the response characteristic of the peripheral auditory system, can then be determined. A computer system containing a high speed analog-to-digital converter, a high-speed clocked pulse counter and a low-pass sharp cutoff filtering system has been designed, built and tested. This system will be used to acquire baseline data on control animals prior to testing.

Experiments to determine the effect of in utero noise exposure on the fetus have continued. The exposure of pregnant CF1 mice to either semi-continuous high level noise (126 dBA jet engine noise) or unanticipated high intensity startling sounds resulted in embryo-lethality and decreased pregnancy maintenance. This effect did not appear to be related to elevation of plasma corticosterone levels. These studies will be repeated in guinea pigs since their audibility curve is very close to that of humans. 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 responses techniques.

A fiber optic lever capable of measuring displacement in the 1Å range across 100 Hz-20 kHz bandwidths has been designed and tested. This system has been optimized for ossicular chain measures. Attempts will be made to make measurements on basilar membrane vibrations in normal and noise exposed animals. Progress has been made in the development of a closed system electroacoustic transducer. This device will be used to present high level wide band minimum distortion sounds to the eardrums of guinea pigs. An instrument which greatly facilitates speech discrimination testing by allowing L eq and peak measures and provides either pink noise, white noise or speech spectrum noise has been designed and constructed.

The effect of protracted noise exposure on the cardiovascular function of primates has been examined via a contract. In these experiments Rhesus monkeys have been exposed for periods of up to the one year to noise conditions resembling the community and workplace. Two animals have been exposed to term (8 months) however the loss of two consecutive control monkeys seriously hinders the interpretation of data at this time. 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. These results seem to implicate changes in the stroke volume rather than an increase in peripheral resistance. In the second phase of this study, stroke volume will be measured directly. In addition other physiological (dPdT, epicardial EKG, cardiac output) and biochemical (blood chemistry, plasma catecholamines and cortisol) parameters will also be monitored.

LIGHT

This program is concerned both with the biological effects of artifical 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, an almost ubiquitous light source found in both the home and work environment, is grossly distorted when compared to sunlight. For this reason, an experiment has begun to determine whether fluorescent lights have any hitherto unknown biological effects. For these studies, C_3H mice have been selected as the test species because they spontaneously develop mammary tumors and because previous studies have suggested that exposure to certain fluorescent lights decrease their lifespan. Preliminary results indicate that 50% of the bred females survived for 46 weeks, 50 weeks and 56 weeks under the daylight, pink and cool white fluorescent lights, respectively. Data on reproduction and pathology are being evaluated.

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 photo-allergy. 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. Laboratory scientists have therefore studied 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 photosensitizing 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 adsorbed 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 adsorption 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

stimulation 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 one adriamycin molecule is bound per 15 nucleotides wherease only one daunorubicinis 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.

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 either mastocytomas or mast cell membranes, although it did increase the number of binding sites available for spin-labeled steric 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) 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, 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 ethrocytes 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 protoporphrin phototoxicity in erthyrocyte membranes and the molecular pathways involved.

Proteins: The binding of Cu^{2+} to human, bovine, porcine, rabbit and rat serum albumins has been measured using a cupric ion selective electrode. These measurements have emphasized the importance of the N-terminal histidine residue for the high affinity binding of Cu^{2+} to serum albumin. The N-terminal turpeptide of human serum albumin (Asp-Ala-His-NH CH_3) has been synthesized. The interaction of this peptide with Cu^{2+}, Ni^{2+} and other divalent metals will be studied using nuclear magnetic resonance. The binding of Cu^{2+} and Ni^{2+} to human serum albumin has also been studied by fluorescence and circular dichroism spectroscopy. With the aid of these techniques, it has been possible to show that penicillamine, a copper mobilizing drug, is a more potent chelator of Cu^{2+} than albumin. Finally, human serum albumin has been cleaved with cyanogen bromide. Work is now underway to characterize the fragments and use them for hg and binding studies.

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 organic hydroperoxy radicals (aunene hydroperoxide, t-butylhydroperoxide). 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 semiquinone-imines; the formation of a polymenic 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.

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. Minoru Inouye (Visiting Fellow) joining the Non-ionizing Radiation Workgroup in April 1980.

Other Activities

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 Non-ionizing 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 Drug Protein Binding, Stockholm, Sweden, March 25-28, 1980, and at the XVI International Congress on Therapeutic Chemistry, Marseille, France, July 9-11, 1980. Seminars at the North Carolina Section of the American Chemical Society, N.C. State University, September 18, 1979 and the Department of Biological Sciences, Ohio State University, Columbus, Ohio, March 12, 1980.

Dr. Reginald O. Cook: Member, Interagency Noise Effects Panel; member Committee on Hearing, Bioacoustics and Biomechanics of the National Research Council, National Academy of Science; member, Working Group 83 on Noise Effects.

Dr. Michael J. Galvin: Adjunct assistant professor, NCSU, Graduate Committee, Cindy Hall, NCSU. Invited speaker, Duke University, May 1, 1980, Membership Committee, Shock Society. Participant, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation.

Dr. B. Kalyanaraman: Invited speaker at the University of Alabama, "Oxygen free radicals", March 25, 1980.

Dr. Teruzo Konishi: Invited speaker International Symposium on Meniere's Disease in Dusseldorf, West Germany.

Dr. Ronald P. Mason: Invited speaker Gordon Conference on "Magnetic Resonance in Biology and Medicine. Invited speaker at CIIT.

Dr. Donald I. McRee: Adjunct Professor, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; 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 task force member by National Telecommunications and Information Administration to develop a documented entitled, "A Coordinated Federal Activities Related to Biological Effects of Non-ionizing Electromagnetic Radiation (0-300 GHz); Elected Board member of the Bioelectromagnetics Society; Appointed member to IEEE's Committee on Man and Radiation (COMAR); Invited Lecturer in Summer School of the Czechoslovakian Society of Biomedical Engineering in Praque, Czechoslovakia; 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.

Dr. Mary J. Ortner: Member of the Animal Experimentation Committee, NIEHS, RTP, N.C.

Dr. Birandra K. Sinha: Presented a seminar at NCI Laboratory of Chemical Pharmacology entitled "Recent studies on the mechanism of action of actinomycin-D and some anthracycline antitumor agents"; member of the Safety Committee NIEHS, RTP, N.C.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50005-06 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	Alec N. Salt	Visiting Fellow	LEB	NIEHS
OTHER:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS

COOPERATING UNITS (if any)

Research Resources 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:
0.7	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)

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 levels of noise and (2) to correlate alteration of the ionic permeability of the cochlear partition with changes in sensitivity of the auditory organ.

FIS-0040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Healthy guinea pigs were exposed to broad band noise for seven days (16 hours on and 8 hours off per day). The overall intensity was 115 dBA. After the period of noise exposure the cochlear potentials were recorded under anesthesia with sodium pentobarbital. The scala vestibuli was perfused with artificial perilymph containing ^{43}K and ^{22}Na. Concentrations of K^+ and Na^+ in the perfusate, perilymph and endolymph were determined by a helium glow photometer and activities of these radioisotopes were measured using a gamma spectrometry system.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potentials. The EP (mean $\pm$ S.D.) in guinea pigs exposed to noise at 115 dBA was 87.9 $\pm$ 3.5 mV which was greater than the value of 84.5 $\pm$ 2.3 mV observed in control guinea pigs.

Following exposure to noise at 115 dBA, the mean ΔCM_{max} (amount of suppression of CM maximum output expressed in dB) and ΔCM_{sens} (elevation of CM sensitivity expressed by sound intensity required to elicit 100 μV peak-to-peak CM) increased by 15 dB and 30 dB, respectively.

The action potential (AP) to 6 kHz tone bursts was more markedly suppressed by noise exposure than CM. The mean loss of the AP, expressed by increment of sound intensity to elicit 100 μV of AP was 40dB.

2. Electrolyte concentrations in the cochlear fluids. In noise exposed guinea pigs, the K^+, Na^+ and Cl^- concentrations in the perilymph were not significantly different from those measured in control guinea pigs. The mean K^+ concentration in the endolymph of guinea pigs exposed to 115 dBA noise was 168.4 mM which was significantly higher than the control value (152.3 mM). On the other hand, a reduction of Na^+ concentration was observed in noise exposed animals. Similar changes in electrolyte concentrations in the peri- and endolymph were observed in noise exposed animals when the scala vestibuli of the cochlea was perfused with artificial perilymph.

3. Distribution of ^{43}K and ^{22}Na in the cochlear fluids. Perfusate containing ^{43}K and ^{22}Na was introduced into the scala vestibuli of the basal turn and only scala vestibuli was perfused for periods ranging from 20 to 90 min in control and noise exposed guinea pigs. In both groups of guinea pigs the activities of ^{43}K and ^{22}Na in samples taken from the scala tympani showed only a few percent of the activity in the perfusate. The concentrations of ^{43}K and ^{22}Na in the endolymph increased as the duration of perfusion was prolonged and in the steady state the normalized concentration of a tracer in the endolymph can be expressed by

$$\frac{{}^*C_{end}}{{}^*C_{peri}} = \frac{C_{end}}{C_{peri}} \{1-\exp(-\lambda t)\}$$

where $*C_{end}$ and $*C_{peri}$ are the concentrations of a labeled ion in the endolymph and perilymph, respectively. C_{end} and C_{peri} are the total concentrations of the ion in the endolymph and perilymph, respectively. λ is the rate constant and t duration of perfusion. The rate constant for K^+ determined with perfusion of scala vestibuli was not markedly different from that obtained in perfusion of both scala vestibuli and tympani.

We plan (1) to continue to carry out the project as outlined, (2) to determine the potential correlation between suppression of end organ sensitivity and membrane permeability changes in noise exposed and ototoxic drug treated animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to various electrolytes has been frequently hypothesized, but the supporting data are rather limited. Present data suggest the possibility that electrolyte movement (especially K^+) across the cochlear partition is involved in the pathophysiological processes underlying noise induced hearing loss. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ear under the influence of physical and chemical agents.

PUBLICATIONS

Konishi, T., Salt, A.N. and Hamrick, P.E. Effects of exposure to noise on ion movement in guinea pig cochlea. Hearing Research 1, 1979, pp. 325-342.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50014-10 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
1.1	0.2	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)

It is the objective of this project to develop 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. A small three-axis electric field probe has been obtained from Narda Corporation and is in the process of being tested for its utility in measuring electric fields in biological material.

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: It has been determined that for the exposure of small biological specimens such as cell systems and isolated neurons, waveguide exposure systems offer excellent characteristics. A waveguide system for exposing isolated neurons to 2450 MHz microwave radiation has been modified and improved. One modification permits the use of mineral oil in the neural stimulating and recording electrode modules so that longer survival times of the isolated nerves can be achieved. A second modification allows the circulation of saline through a constant temperature water bath into the waveguide exposure chamber. This modification enables us to maintain a constant temperature of both the control and exposed nerves during various power density levels of irradiation. A third modification to the system will allow the sampling of fluid around the nerve in order to evaluate ionic transport.

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 pulsewave irradiation.

A small three axis electric field probe designed by the Bureau of Radiological Health and fabricated by Norda Corporation has been obtained. This probe should be capable of measuring the electric field intensity inside biological material. The probe is currently being evaluated for its accuracy and utility as an instantaneous dosimeter.

Waveguide exposure systems will continue to be fabricated, calibrated, and operated during the next fiscal year. The new systems will provide the institute with the capability of exposing isolated neurons, cell systems and macromolecules to not only continuous wave, but other wave forms such as pulsed and frequency modulated waves. In addition, the new system will allow us to expose isolated mammalian nerves which are difficult to maintain in a viable state outside the animal body. The development of a system to measure the electrical properties, dielectric constant and conductivity, of biological material will continue.

SIGNIFICANCE TO BIOMEDICAL 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.

PUBLICATION

McRee, D.I. and Wachtel, H.: Long-term, low-level exposure of marine animals. Radio Science, 14: 75-79, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-06

PERIOD COVERED

October 1, 1979 to September 30, 1980

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:
0.4	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 objective of this project is to determine the effects of microwave radiation on neurological response. Isolated neurons such as the abdominal ganglion of the Aplysia, lobster ganglia, the sciatic nerves of frogs and the saphenous nerves of cats will be exposed to CW, pulsed, and modulated microwave radiation in the specific absorption rate range (SAR) of 0 to 100 mW/g. Fatigue and recovery of the neurons under different modes of stimulation will be studied. The basic mechanisms of interaction will be investigated in cases where changes occur. Experiments to determine if continuous and pulse wave, 2450 MHz microwave radiation increase the fatigue rate or change the vitality of the frog sciatic nerve were completed. Distinct changes in the vitality and refractoriness of the exposed nerve were seen in comparison to the control nerves for SAR's of 10 mW/g and above. No differences in rundown time were observed between the continuous wave and pulse wave exposures using the same average SAR. Exposure of oubain treated nerves eliminated the difference in loss of vitality between the control and exposed nerves. This result indicates that the microwaves interfere with or perhaps inhibit the Na-K pump.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the sciatic nerve of the frog, lobster ganglia, abdominal ganglion of the Aplysia, and saphenous nerves of cats will be exposed to CW, pulsed and modulated microwave radiation in the specific absorption rate (SAR) range of 0-100 mW/g. The effects of the radiation on nerve function and the mechanisms of interactions involved in any changes will be investigated.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of isolated frog sciatic nerves to pulsed 2.45-6Hz 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 or changed the threshold for stimulation of the nerves, a peak power of 4 kW and a pulse width of 10 msec was 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 msec apart with an average SAR of 10 mW/g. No differences in rundown of the nerves over that obtained with continuous 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.

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 need for significant effort in this area. At present, the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

PUBLICATION

McRee, D.I. and Wachtel, H.: The effects of microwave radiation on the vitality of isolated frog sciatic nerves. Radiation Research (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50017-07 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	M. Inouye	Visiting 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:
0.8	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)

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/cm² (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 $\times 10^3$ cells/mm³). No other indices were significantly different.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHOD EMPLOYED: The objectives of this project are 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).

MAJOR FINDINGS AND PROPOSED COURSE: 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 ($P < F0.01$) in the exposed males. Spermatozoal viability and several morphological characteristics in the exposed birds were not consistently different from controls. Relative testis 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.

This study was conducted to assess reticuloendothelial (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 ($t_{\frac{1}{2}}$ 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.

Another group of fertilized Japanese quail eggs has been exposed to 2.45-GHz CW radiation at 5 mW/cm^2. The quail have been placed in regular feeding and housing facilities for 6 weeks. At 6 weeks of age, quail of both sexes were examined for humoral and cellular immune responsiveness, reticuloendothelial system activity, and tissue and plasma enzyme levels. Brains were weighed and histological preparations of brain tissue were made. The data from these experiments are presently being analyzed. Additional research to investigate the effects of microwaves on brain development in the Japanese quail will be carried out.

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. 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 repeated as described above.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-05 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

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

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.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.

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 by 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.

MAJOR FINDINGS AND PROPOSED COURSE: This project has suffered from hardware interface problems 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 and numerous iterative changes, initial experiments are not planned until several smaller projects have been completed -- probably late fall 1980.

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 perceive d in a noisy environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-04 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Optical Fiber Motion Detector 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
		1. Biological Science Research Division 2. Frank Porter Graham Research Division 3. Center for Alcohol Studies

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 be the area of more efficient illumination sources 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 has been prepared.

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 m 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 optic levers offers an attractive alternative in some shock and vibration problems, particularly where knowledge of displacement waveform characteristics is desirable and in high frequency applications.

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 qualify 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 commercially available models. Since the theoretical relationships have been developed for both laser and Lambertian sources and confirmed by measurement, it is now possible to accurately predict the ultimate resolution for given fibers, bundle configurations and illumination sources.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50022-04 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 prematurally 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. 10-76)

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.

MAJOR FINDINGS AND PROPOSED COURSE 1) The pneumatic drive mechanism under test appears to have solved the failure rate problem associated with the earlier solenoid operated device. Previous tests with animals indicated not physiological parameters associated with hearing impairment (CM, AP) deteriorated in different ways when exposed to equal energy impact vs. continuous noise. Pilot tests involving animals will begin anew in July.

2) The tone burst generator was designed and constructed and is currently 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 devices was designed and constructed and is currently being 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 a few, if any, well controlled laboratory studies of hearing damage from a type of noise which prevades 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 psychophysical 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, pre-distortion, 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.

This project report combines several additional projects with previous Project #Z01 ES 50021-02 EB and Z01 ES 50022-03 LEB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50026-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 D. 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)

For some time, the ability to perform experiments requiring the presentation of high level, wide band, minimum distortion sounds to the eardrum of guinea pigs has been unduly restricted by presently available small electroacoustical transducers suitable for earbar interface. As a result, high level sounds have had to be produced from loudspeakers mounted some distance from the ear (field stimulation). As a result, distortion, maximum achievable level and accuracy of measurement suffer. The availability of electroacoustic transducers operated into closed systems (earbar or ear canal) and capable of producing very high levels would allow one time acute exposures at levels sufficient to cause immediate auditory damage resulting in significant time savings. In addition, the pseudorandom noise which will serve as stimulus in some experiments, is characterized by a line spectrum. Prevention of distortion in line spectrums is particularly important. There have been reports in the high fidelity literature of earphone transducers having many of the desirable characteristics, so an attempt will be made to have such a device optimized for closed system applications.

PROJECT DESCRIPTION

METHODS EMPLOYED: Very sophisticated level, frequency and distortion tests of any electroacoustics transducer can be performed in our laboratory. Devices will be built to our specifications by others.

MAJOR FINDINGS AND PROPOSED COURSE: Small commercially available electroacoustic transducers are optimized to operate into an acoustic impedance simulating that of the human ear canal and pinna, (6 ml). A change in this volume of as much as 10% causes significant frequency response and distortion changes. The acoustically compliant volume of conventional ear bars and guinea pig ears is about 0.5 ml. Mass and resistive loading are negligible. Manufacturers will be approached in an effort to get an optimized prototype built to our specifications. Little was accomplished during the past year on this project, therefore we are discontinuing this project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Sound sources with which precise control (i.e., no distortion) of acoustic waveforms at the ear, across wide band width and amplitude can be produced. It is believed that current electroacoustic transducer technology can be exploited to achieve advances in noise exposure research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50027-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Prenatal Exposure to High Noise Levels on Auditory Thresholds in Guinea Pigs

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
	Alec Salt	Visiting Fellow	LEB NIEHS

COOPERATING UNITS (if any)

Laboratory of Environmental Toxicology

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:
.8	.5	.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)

Pregnant guinea pigs were exposed to tape recorded textile mill (weaving room) noise at 115 dB Sound Pressure Level, for 8 hours/day, 5 days week. Exposure generally began at the third trimester of pregnancy, but varied due to discrepancies between the estimated breeding date and the actual breeding date as determined by delivery. The hearing levels of offspring were measured and compared with that of offspring from otherwise identical mothers. Brainstem evoked responses recorded from chronically implanted scalp electrodes showed deterioration in the hearing of the offspring from exposed mothers. These differences appear significant at the $p < .01$ level.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant guinea pigs were exposed to tape recorded weaving room noise in an acoustically shielded chamber, 8 hours per day, 5 days per week. The hearing levels of offspring were tested on either 2 or 3 sequential occasions by the noninvasive sound evoked brain-stem response (BSER) and selected animals from each litter were tested by measurement of the N_1 action potential and by cochlear microphonics before sacrifice. Temporal bones (cochleas) were saved for subsequent histological analysis. BSER techniques involve computer averages of thousands of stimuli/responses rapidly.

MAJOR FINDINGS AND PROPOSED COURSE: Hearing levels of the offspring from guinea pigs exposed to high level 115 dB (150 Hz 8 kHz) textile mill noise during latter third of pregnancy were found to differ consistently from the hearing levels of offspring from non-exposed but otherwise similar females. Hearing was measured in all animals by brainstem evoked responses and additionally by the cochlear microphonics and N_1 action potential (from one animal per litter). Hearing level differences on the order of 5-10 dB (measured by BSER) were found significant at the $P < .01$ level. Results are similar to those of Daniel, a Polish researcher, who found hearing levels of the offspring from exposed mothers to be worse than controls. There were some, perhaps significant, differences in the two studies: Pregnant females were exposed full term in the Polish study and hearing was measured by a rather crude suprathreshold behavioral technique.

This project afforded the opportunity to compare computer analyses of averaged BSER data in several ways: RMS, 5th peak amplitude, 5th peak latency, and entire waveform time lag correlation. The latter in particular is much easier to perform, would provide significant advantages in small animal testing, because latency differences are very small and difficult to measure relative to humans.

SIGNIFICANCE 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. One indication of this concern was the request made by the Air Force to the National Academy of Sciences Force that they convene a scientific panel to assess that risk. The panel has not yet reported its findings.

In 1977 Bock and Sanders presented evidence for the existence of a period of enhanced susceptibility to auditory system damage from noise insult in hamsters. The inception of this critical period corresponded to the beginning of structural maturation of the auditory system (approximately day 20 after birth in hamster) and continued for about 25-30 days. Because of the very short fetal stage in hamsters (18 days), the corresponding stage occurs prenatally in humans. Previously Falk, Cook and Haseman have

presented histological, evidence for enhanced auditory damage susceptibility of very young versus older guinea pigs. Recently Daniel, in a 1976 study in Poland, reported that the offspring of pregnant guinea pigs exposed to weaving mill noise (105 dBA) had higher threshold levels (poorer hearing) than the offspring of mothers not so exposed. Because of the comparatively long fetal period of guinea pigs (68 days), the period corresponding to auditory system structural maturation also occurs prenatally. The results of this study provide additional suggestive evidence for a critical susceptible period in the development of the auditory system. This study is complete.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50028-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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)

It has been demonstrated that changes in ionic permeability of the endolymph/perilymph barrier are a significant factor in the hearing loss produced by noise or ototoxic antibiotics. The present study has examined the physiological effects on the cochlea of two drugs, amiloride and tetraethylammonium (TEA) which are reported to reduce membrane permeabilities to sodium and potassium ions respectively in a variety of tissues. The application of TEA to cochlear endolymph produced effect similar to noise exposure. Cochlear microphonics (CM) were reduced, endocochlear potential (EP) was increased and the rate of EP decline during anoxia was decreased. Application of amiloride to the cochlea did not affect CM or EP. These results support the view that normal potassium permeability properties of the endolymph/perilymph barrier are essential for normal cochlear function.

PHS-6949

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs weighing 300-500g were anesthetized with sodium pentobarbital (30-35 mg/kg) and the right cochlea exposed. Endocochlear potential (EP) was recorded from the basal turn with glass electrodes. Cochlear microphonics (CM) and action potentials (AP) were recorded from differential electrodes in scala vestibuli and scala tympani. Tone-induced responses were generated by 6 kHz tone bursts and 72 dB SPL.

The drugs amiloride or TEA were applied to the cochlea by one of two methods:

a) Perfusion of the perilymphatic space.

The drug was dissolved in a solution similar in ionic composition to perilymph and was injected at a rate of 2 µl/min into the basal turn of scala tympani. Fluid was allowed to escape from scala vestibuli. The duration of perfusion was normally 40 minutes.

b) Iontophoresis into endolymph.

Single barrel glass electrodes with their tips broken to 2-3µ were filled with solution containing the dissolved drug. Electrodes were inserted into scala media and a pulsed current of 3×10^{-7} A (500 msec pulse width, 1/sec) was passed to eject the positively charged, ionized drugs. The duration of iontophoresis was 20 mins.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Perilymphatic application. Perfusion of the perilymphatic space with solution similar in composition to perilymph (control perfusion) produced slight changes in the CM, AP and EP. The mean changes ($\pm$s.d.) observed in 6 animals were -9.3 $\pm$ 9.8% + 40 $\pm$ 19.1% and + 2.6 $\pm$ 2.0 mV for the CM, AP and EP, respectively. Addition of 10^{-3} M amiloride to the perfused solution had an insignificant (5% level) effect on the CM and Ep, the mean changes observed in 6 animals being -20.8 $\pm$ 7.33% and +0.6 $\pm$ 1.61 mV. The AP response was suppressed by amiloride at this concentration, the mean response falling by 65.1 $\pm$ 16.1%. TEA at a concentration of 10^{-2} M produced effects similar to amiloride during 40 mins perfusion. The mean changes of CM and EP in 4 animals were -2.6 $\pm$ 4.5% and + 3.4 + 2.5 mV, which were not significantly different from control values. The AP was suppressed by 60.0 $\pm$ 25.9%. These results demonstrate that both drugs interfere with neural transmission, as indicated by the AP suppression observed. However, the stability of CM and EP during drug treatment indicates that either the drugs do not influence hair cell function or that they do not have access to the region of the cell on which they are effective.

2) Endolymphatic application. Iontophoretic injection of potassium (control iontophoresis) for 20 mins produced small changes in CM, AP and EP similar to those produced by perfusion. The mean changes ($\pm$ s.d.) in 6 animals were -4.6 $\pm$ 6.5%, + 1.5 $\pm$ 8.6% and + 1.5 $\pm$ 1.5 mV for the CM, AP and

EP, respectively. Iontophoretic application of amiloride from an electrode containing 160 mM KCl saturated with amiloride HCl (approx 5 mM) produced no greater changes than did the control procedure. The mean changes of CM, AP and EP in 3 animals were -5.2 ± 4.2%, 0.9 ± 3.6% and + 2.2 ± 2.0 mV, respectively. However, iontophoretic injection of TEA from an electrode containing 160 mM TEA produced significant changes in both CM and EP. In 8 animals, the mean CM was reduced by 36.0 ± 19.4% while the EP increased by 9.5 + 3.1 mV. AP was not changed significantly, the mean change being 1.1 ± 9.2%. When 4 TEA treated animals were subjected to anoxia, the EP decreased from a mean value of 97.5 mV to -2.0 mV during 5 minutes. This was significantly slower than the rate observed in 3 control animals, in which the EP declined from 88.6 mV to -31.8 mV during 5 minutes.

With the exception of the AP response, the response changes produced by TEA iontophoresis into endolymph were qualitatively similar to the effects produced by intermediate level noise exposure. The lack of AP suppression observed may have been due to the TEA being applied locally to a small region while broad band noise exposure affects the entire length of the cochlea. These data support the concept that movement of potassium ions between endolymph and the hair cell contributes significantly to the hair cell response while the movement of sodium ions appears to play a less important role.

Although a potassium permeability change of the endolymph/perilymph barrier appears to be an important factor in noise and ototoxic antibiotic induced hearing loss, the actual site of the change cannot be ascertained by the usual methods of extracellular recording. We, therefore, plan to use ultrafine ion-selective electrodes with tip diameters less than 0.5 μ to explore the electrochemical profile of the organ of Corti. Comparisons will be made between the profiles found in normal and noise or drug traumatized cochleas. It is thought that this study will localize more closely the site at which the noise induced permeability change takes place.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Although the characteristics of noise-induced hearing loss have been well documented, the mechanisms of response suppression and subsequent hair cell loss have yet to be established. Our data demonstrate that a major factor in noise-induced hearing loss may be a reduction in the potassium permeability of the endolymph/perilymph barrier. This represents an important step in our understanding of the physiology of noise trauma.

PUBLICATIONS

Salt, A.N. and Konishi, T.: Effects of noise on cochlear potentials and endolymph potassium concentration recorded with potassium selective electrodes. Hearing Research 1, 343-363 (1979).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50029-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Acridine Spin Labels as Probes for Nucleic Acids and Their Protein Complexes

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:
0.45	0.45	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 design spin label probes which can be used to study the binding of mutagens to nucleic acids. Since acridines are mutagenic and bind strongly to nucleic acids, we have designed and prepared acridine spin labels containing the stable nitroxide free radical. The binding of these acridine spin labels to nucleic acids has been studied by electron spin resonance. These spin label derivatives bind to nucleic acids by intercalation and have high base specificity. However, this specificity is not absolute. We have used these labels in studying the binding of histone H_1 to DNA. Our studies suggest that H_1 binds to the A-T-rich bases of DNA in the major groove.

PHS-0046

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Our earlier studies have shown that the 9-amino acidine spin label derivatives (I and II) bind to DNA. The binding of these labels to DNA was characterized using ESR, thermal denaturation and sedimentation coefficients determinations. These studies had indicated that the labels were intercalated into DNA. In addition to intercalation, ionic interactions at the phosphate groups and the functional grooves of DNA were also involved. Furthermore, our ESR studies with denatured DNA suggested that these labels could be used for distinguishing between single-stranded and double stranded structures.

The binding of the DNA was characterized by using various synthetic polynucleotides. We had shown that while Label I was bound at G-C-rich regions, label II interacted with A-T-rich regions of DNA. Using ESR technique, we had shown that histone H_1 interacted primarily at the A-T-rich regions in the major grooves of DNA.

SIGNIFICANCE TO BIOLOGICAL RESEARCH AND PROGRAMS OF THE INSTITUTE: In conclusion, we have shown, using ESR techniques, that there are at least three sites for acridines in DNA. These are: (a) ionic binding at the negatively charged phosphate groups; (b) ionic binding at the major and minor groups; and (c) intercalation into the bases of DNA. Such interactions of acridines with DNA may have significance in their mutagenic properties.

This project is essentially complete and no future work is planned at this time.

PUBLICATION

Sinha, B.K., Chignell, C.F. and Wee, V.T.: Structural investigations DNA-histone complexes. A Spin label study. Nucleic Acids Res., 6, 3703-3713, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50030-02 LEB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.45	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 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

MAJOR FINDING AND PROPOSED COURSE: We have synthesized N^2[4-(2,2,6,6-tetramethyl-1-piperidinyloxyl)] 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. 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 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 polymer 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 $O_2^-/H_2O_2/OH$, and membranes 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 mutagenecity, 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.

PUBLICATIONS

Sinha, B.K., Cox, M.G., Chignell, C.F., and Cysyk, R.L. Synthesis and Biological properties of N^2-substituted spin-labeled analogs of actinomycin D. J. Med. Chem. 22, 1051-1055 (1979).

Sinha, B.K. and Cox, M.G. Stimulation of superoxide formation by actinomycin D and its N^2-substituted spin-labeled derivatives. Mol. Pharmacol. 17, 432-434 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50031-03 LEB

PERIOD COVERED
October 1, 1979 - September 30, 1980

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	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:
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 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

PHS-6040
(Rev. 12-76)

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-doxylstearic 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 investigated 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 6-10^5 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, the R-48/80 bend 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 through fluorescence microscopy that 48/80 binds to the outer membrane at low concentrations and to inner constituents at higher levels. We are currently expanding our studies to include the effect of 48/80 on the proteins of ghost membranes as studied by circular dichroism. We also plan to study individual proteins in their solubilized form using the same procedure.

The studies should contribute to our knowledge of the apparent protein denaturation caused by 48/80. We are also continuing our studies using the spin labeled maleimide ghosts. By studying the effects of 48/80 on membrane proteins directly we hope to characterize more precisely its molecular interaction. We intend to expand our fluorescence microscopy studies to determine the effect of heat treatment and trypsin on the binding of 48/80. Furthermore we will also study the effects of pharmacological treatments such as colchicine and cytochalasin B and also drugs which are known to compete for 48/80 binding sites such as polymyxin B. We are therefore continuing the work of our previous year in order to identify the binding site and molecular mechanism of 48/80. Little was accomplished during the past year on this project, so we are continuing the project another year.

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, dextron, calcium conaphores 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 conjunctivities 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.

PUBLICATIONS

Ortner, M.J., Sik, R.H., Chignell, C.F. and Sokoloski, E.A.: A nuclear magnetic resonance study of compound 48/80. Mol. Pharmacol. 15: 179-188, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50032-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
1.0	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 binding of metal ions, (copper and nickel, in particular) to albumin from different animal species was studied using different physical techniques. The action of D-pencillamine, a copper mobilizing drug, on copper binding to HSA was studied. HSA was labeled with fluorescent labels. In addition, studies are being carried out with synthetic peptide and chemical and enzymatic fragments of HSA. Attempts will be made to characterize the copper binding sites, in addition to the N-terminal site.

PHS-6040
(Rev. 10-75)

PROJECT DESCRIPTION

METHODS EMPLOYED: A cupric ion selective electrode was used to measure the parameters of copper binding to albumins from different species. The binding of Cu^{2+} and Ni^{2+} to HSA was also studied using circular dichroism. Chemically modified and spectroscopic labeled HSA and fragment peptides are being studied with fluorescence and circular dichroism techniques. Both tri- and tetra peptides with N-terminal sequence of HSA are being synthesized. Nuclear magnetic resonance methods will be employed to study the conformation and characterization of the metal ion and drug binding sites of HSA and its chemical/enzymatic fragments.

MAJOR FINDINGS AND PROPOSED COURSE: The parameters of binding of copper to albumins of different species were determined using ion-selective electrode. The number of binding sites determined are 3.7 ($Ka = 3.14 \times 10^6\ 1M^{-1}$) and 12.7 ($Ka = 2 \times 10^4\ 1M^{-1}$) for bovine, 3.9 ($Ka = 2.8 \times 10^6\ 1\ M^{-1}$) and 15.1 ($Ka = 7.5 \times 10^{-3}\ 1M^{-1}$) for rabbit, 2.62 ($Ka = 1.8 \times 1\ 10^6\ 1M^{-1}$) and 26.4 ($Ka = 43 \times 10^3\ 1M^{-1}$) for rat and 1.86 ($Ka = 4.1 \times 10^5\ 1M^{-1}$) and 18.0 ($Ka = 5.8 \times 10^3\ 1M^{-1}$) for porcine albumins. The binding parameters for bovine albumin are in agreement with those determined previously. The relative affinities of copper for high affinity sites are in good agreement with the data previously published by Callan and Sunderman for the binding of nickel.

The circular dichroism of the d-d transitions was observed on addition of Cu^{2+} and Ni^{2+} to albumins under different conditions. In the absence of any pH adjustments, the ellipicites at 570 (-) and 480 (+) nm increase on addition of Cu^{2+} to HSA. However, some minor differences have been observed between copper binding to HSA and BSA. The ellipticity at 570 and 480 nm increases steadily till the HSA to Cu^{2+} ratio is 1 to 3. Further increase in copper equivalents doesn't show any increase. The effect of N_3^- on the bonding of Cu^{2+} to HSA was studied by CD. No ligand induced absorption was observed till the N_3^-: HSA is 2 to 1. Further increase in N_3^- equivalents exhibits transitions that increase linearly below 350 nm. The Ni^{2+} binding to HSA was studied at pH 7.4 and 10.4. The amplitude of the trough at 475 nm increases there are 4 equivalents of Ni^{2+} to HSA. However, at pH 10.4, the trend is slightly different. The effect of D-pencillamine, a copper mobilizing drug administered to arthritic patients, on the binding of Cu^{2+} and Ni^{2+} was also studied. From the CD studies, it seems that there are about three copper binding sites which have comparable geometry. Pencillamine is more potent as a copper chelator than albumin and this probably is the reason for its mechanism of action.

HSA was labeled by pyrene maleimide, a fluorophore. This resulted in cross-linking of the N-terminal with the lone cysteine SH group. The induced dichroism of the bound label indicated a chiral environment for either the N-terminal or the SH - group or for both. The induced dichroism of the defatted and fluorescent labeled HSA enhances with progressive addition of fatty acids. Copper and fatty acid labels were found to quench the fluorescence of bound fluorophore. Attempts will be made to make some distance measurement

from the label to some of the drug binding sites. Attempts to couple the protected aspartic acid to Ala-HisNHCM$_3$ to make a tripeptide with the N-terminal sequence of HSA has recently met with some success. Synthesis of a tetrapeptide Asp-Ala-His-Lys (N-terminal sequence of HSA) is also being attempted to explore if ε-amino group of Lys plays any role at the N-terminal copper binding site. Once the synthesis is complete, it is planned to study the geometry of this site using cryslattographic and NMR probe techniques.

HSA was subjected to CNBr cleavage and the fragments are being isolated. The fragments will be used for NMR studies in fingerprinting. Proton and Carbon-13 NMR studies of these fragments and HSA may provide some idea on the conformation and dynamics of HSA in solution. Besides, it will enable the characterization the binding sites of different drugs.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50033-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Do Nitroaromatic Free Radicals Covalently Bind to Macromoleculesμ

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
	Carl Polnaszek	Research Chemist	Mpls. VA Hosp.
	J.L. Holtzman	Pharmacologist	U. of Minn.

COOPERATING UNITS (if any)
Department of Pharmacology, University of Minnesota

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We have proposed that nitroaromatic anion free radicals (RNO_2^-) covalently bind to glutathione, protein or to other tissue macromolecules,

$$RNO_2^- + P \longrightarrow RNO_2 - P$$

where P represents a macromolecule or glutathione. In most studies on the covalent binding of reductive intermediates of nitro compounds to protein it is usually assumed that the hydroxylamine metabolite is the species that covalently binds. Yet there is only very circumstantial evidence to support this assumption. Since as much as 14% of a reductive intermediate of some nitrofurans becomes covalently bound to protein, it should be possible to observe spectroscopically the covalent binding of the anion radicals, if this intermediate is indeed responsible for binding. Because reduced glutathione depletion is thought to be important in some drug toxicities, it is important to determine if the anion radical directly bind to glutathione. In addition, L-cysteine or GSH decreased the covalent binding of one nitrofuran carcinogen by 99.9% without affecting the microsomal nitroreductase activity. Just recently the binding of a reductive metabolite(s) of two nitrofurans to DNA and RNA has been reported.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) has been utilized to detect steady-state levels of nitrocarcinogen free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: We have examined the effect of added glutathione, protein, DNA and RNA on nitrofuran anion radical kinetics. The steady-state concentration of anion radical as determined by an ESR spectrometer, will be altered by protein concentration if the radical binds to protein. Covalent binding of $\dot{RNO}_2^-$ to glutathione, protein, or other macromolecules, P, will compete with the disproportionation of the radical as a decay mechanism according to the following rate expression,

$$\frac{d[\dot{RNO}_2^-]}{dt} = k_1 [E] [RNO_2] - 2k_d [\dot{RNO}_2^-]^2 - k_c [\dot{RNO}_2^-] [P],$$

where k_c is the rate of covalent binding by the anion radical to protein, and $k_1 [E]^c [RNO_2]$ is the rate of enzymatic radical formation.

$$\dot{RNO}_2^- + P \xrightarrow{k_c} RNO_2 - P$$

The steady state condition yields

$$[\dot{RNO}_2^-]_{ss} = \frac{-k_c [P] + \sqrt{k^2_c [P]^2 + 8k_d k_1 [E] [RNO_2]}}{4k_d}$$

Any reversible association between P and E or RNO_2 will be reflected in k_1. If k_1 is unchanged by increasing P, but the steady state radical concentration is reduced by increasing P, then $\dot{RNO}_2^-$ is probably covalently binding to P.

Work in Dr. Holtzman's laboratory indicates that 100 mM GSH does not affect the steady-state concentration of a nitrofuran carcinogen free radical in microsomal incubations. Published work indicates that the GSH does not affect microsomal nitroreductase activity, but can decrease protein binding by 99.9% at 0.4 mM. This result indicates that $\dot{RNO}_2^-$ is not the reductive metabolite(s) that covalently binds to protein. Neither DNA nor bovine serum albumin decrease the free radicals concentration. $\dot{RNO}_2^-$ does not appear to react with GSH, protein, or DNA. On the other hand, RNA (8 mg/ml) decreased the radical concentration by 50% in a concentration dependent fashion. The rate of reduction of the nitrofuran carcinogen by xanthine oxidase was increased from 7.98 ± 0.13 nmole/min with RNA. These data suggest that the anion radical reacts with RNA. This project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nitro carcinogens, five of which are available from NCI's chemical repository, are thought to require reductive activation before they can initiate tumor formation. The binding of a reductive intermediate to tissue macromolecules is thought particularly important in this regard. The investigation of the possible reactions between $\dot{RNO}_2^-$ and GSH or macromolecules contributes to our understanding of the mechanism of nitro activation.

Our results support the conclusions of other workers, who have proposed that the reactive intermediate is the hydroxylamine metabolite. Although the chemical structure of the covalently binding metabolite is still unknown, our work has eliminated the nitro radical anion as a possible candidate.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50034-03 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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)

With one exception all mammalian nitroreductases are markedly inhibited by oxygen. Previous invenstigations 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 10^5, whereas dicoumarol inhibited 94^5. 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.

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.

PUBLICATIONS

Peterson, F.J., Mason, R.P., Housepian, J. and Holtzman, J.L.: Oxygen-sensitive and insensitive Nitroreduction by E coli and Rat Hepatic Microsomes. J. Biol. Chem. (In Press).

Peterson, F.J., Mason, R.P., Horsepian, J. and Holtzman, J.L.: One- and two-electron reduction of nitrofurazone by microsomal and E. coli nitroreductases. J. Biol. Chem. 254: 4009-4014, 1979.

Perez-Reyes, E., Kalyanaraman, B. and Mason, R.P.: The reductive metabolism of metronidazole and rondiazole by aerobic liver microsomes. Mol. Pharm. 17: 239-244, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50036-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

A Search for Free Radical Metabolites of Polycyclic Hydrocarbons

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

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)

We propose to use electron spin resonance to search for free radical metabolites of polycyclic hydrocarbons and dibenzodioxins. Free radicals of electron donor molecules are formed in the peroxidase reaction according to the following mechanism.

$$\text{Peroxidase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O$$

$$\text{Compound I} + R \longrightarrow \text{Compound II} + R^+$$

$$\text{Compound II} + R \longrightarrow \text{Peroxidase} + R^+$$

Many compounds are known to undergo such a one-electron oxidation in the presence of a variety of peroxidases and hydrogen peroxide. Electron spin resonance spectra have been obtained of the free radical metabolites of hydroquinone and N-hydroxy-2-acetylaminofluorene and many biological compounds. This class of enzymes appears to have little substrate specificity, any electron donor with a sufficiently low oxidation potential should be metabolized to a free radical. In addition to lacto-peroxidase and myeloperoxidase, hemotin, methemoglobin and cytochrome P-420 all have peroxidase activity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) has been utilized to detect free radical intermediates. Difference absorption spectra have been used to follow the formation and decay of compound I and II.

MAJOR FINDINGS AND PROPOSED COURSE: Ten μl/ml of 30^{5} H_2O_2 was added to an incubation mixture containing horseradish and the tetrasulfonic acid derivative of pyrene in acetate buffer (0.2 M pH 4.6). The incubate was then aspirated through a stainless steel needle into an aqueous flat cell mounted in the microwave cavity of the ESR spectrometer. The ESR spectrum at room temperature was immediately determined. The exploratory investigations on pyrene and benz[a]pyrene were unsuccessful in that an ESR spectrum was not observed. Furthermore no color change was observed as would be expected if benz[a]pyrene was being oxidized to a quinone. Quinone products would be expected in this free radical pathway. A review of the literature did not reveal any reports of polycyclic hydrocarbon oxidation by peroxidases, so this project was concluded.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolic activation of aromatic hydrocarbons by one-electron oxidation has been proposed to be of importance in the mechanism of tumor initiation, but no one has presented evidence for the enzymatic formation of the proposed cation free radicals. The formation of the analogous cation free radical of 2,3,7,8-tetrachlorodibenzo-p-dioxin has also been postulated as the oxidative metabolite which binds to rat liver microsomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50037-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effect of 2450 MHz Microwaves on Plasma and Tissue Enzymes of Guinea Pigs

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:
.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)

Project discontinued, see project Z01 ES 50038-02 LEB, which describes work on influence of microwave radiation on cardiovascular system.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50038-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
0.5	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)

The objectives of this project are to determine the influence of microwave radiation on cardiac tissue in vitro and in vivo. The influence of microwave radiation during embryogeny on the development of cardiac tissue was determined. No effects were noted on the morphological or biochemical parameters measured for Japanese quail myocardium in embryos exposed to 5 or 20 mW/cm² during the first 8 days of development. Techniques for exposing isolated rat atria to microwave radiation have been developed. The rate and force of contraction will be monitored and the responsiveness of the tissue to drugs during microwave exposure will be determined. Also, certain biochemical and physiological parameters which are indicative of cardiac function will be measured in unanesthetized rats during whole body exposure to 2450 MHz microwaves. The parameters measured include plasma creatine phosphokinase activity, electrocardiogram and blood pressure during 4 hours exposure to microwaves at 5 and 10 mW/cm². No data is available on these two areas of study.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: a. This experiment was designed to examine the effects of microwave radiation on the cardiogenesis of Japanese quail embryos, exposed during the first days of development to 2.45 GHz CW microwaves at incident power densities of 5 or 20 mW/cm^2.

b. Isolated rat atria will be exposed to microwave radiation at specific absorption rates of 1, 10 and 100 mW/g for periods up to 5 hours. During exposure, rate and contractile force will be monitored. In addition, the response of the tissue to drugs will be determined during microwave exposure.

c. Whole bodies of adult, male rats will be exposed to microwave radiation of 5 and 10 mW/cm^2 at carefully temperatures and exposure levels. Using a specially designed irradiation chamber, rats will be exposed either dorsally or ventrally, and certain hemodynamic (blood pressure, heart rate, electrocardiogram) and biochemical parameters will be measured following 4 hours exposure.

MAJOR FINDINGS AND PROPOSED COURSE: The results show that neither exposure level was capable of inducing changes in either the morphology of the embryonic heart, or the ultrastructure of the myocardial cells. A comparison of the enzymatic activities of lactate dehydrogenase (LDH), glutamic oxaloacetic transminase (GOT) and creatine phosphokinase (CPK) failed to reveal any statistical differences between the non-exposed controls and those groups exposed to either 5 mW/cm^2 or 20 mW/cm^2. The values obtained for each enzyme expressed relative to 100 μg protein was as follows: LDH, 650 Wroblewski units; GOT, 120 Karmen units; CPK, 275 International units. The data indicate that 2.45 GHz microwave irradiation at 5 or 20 mW/cm^2 has no effect on the measured parameters of the Japanese quail myocardium during the first eight days of development.

The studies described in the second and third paragraphs will be continued. An additional study on microwave interactions with cardiovascular system is described in Z01 ES 50040-03.

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. and D.I. McRee. Effect of 2.45 GHz Microwave Radiation on Embryonic Quail Hearts. Bioelectromagnetics (Accepted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50039-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	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:
0.1		

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 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.

PHS-6040
(Rev. 10-76)

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 simulatenous with microwave exposure. The drug-induced histamine release was terminated by addition of antimycin to the cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: 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 te health effect of physical factors in the environment.

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, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50040-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
.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)

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 preliminary results suggest 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 of 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 38-02 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50041-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
0.7	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)

Ionic permeability changes in the endolymph-perilymph barrier are significant factors in the physiological mechanisms underlying the noise-induced hearing loss. The purpose of this study is to estimate membrane permeability in normal and pathological cochlea by measuring alterations of the passive K^+ efflux from the endolymph and the electrochemical potential difference between endolymph and perilymph during anoxia.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs anesthetized with sodium pentobarbital were used. The sound induced responses were recorded with differential electrodes placed in the basal turn of the cochlea. 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 electrodes. When the active ion transport is abolished by anoxia, K^+ ions diffuse passively from the endolymph to perilymph. If the volume of the endolymph remains 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 expressed by

$$G_K = \frac{V_{end} F \frac{d[K^+_{end}]}{dt}}{\frac{RT}{F} \ln \frac{[K+_{end}]}{[K+_{peri}]} + \Delta\psi}$$

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 was treated with kanamycin 400 mg/kg for 3 weeks guinea pigs were tested 2 weeks after treatment. A second group was exposed to broad band noise at 115 dbA for periods ranging from 11 to 15 days. A third group was used as control animals.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear potentials. The CM_{sens} was reduced to 88.8 $\pm$ 5.3dB SPL and the CM_{max} was suppressed to 370 $\pm$ 90 μV in noise exposed guinea pigs. The CM was markedly suppressed in kanamycin-treated guinea pigs. CM_{max} in all cases being less than 80 μV. The preanoxic values of the EP were 92.1 $\pm$ 3.9 mV and 91.4 $\pm$ 5.0 mV kanamycin treated guinea pigs and noise exposed guinea pigs respectively, which were significantly higher than the EP recorded in control animals.

2. K^+ conductance and K^+ permeability coefficient of the endolymph-perilymph barrier. The preanoxic values of K^+ electrochemical potential difference were 189.6 ± 4.9 mV, 190.4 ± 5.9 mV and 203.2 ± 6.2 mV in control, noise exposed and kanamycin treated guinea pig respectively. The electrochemical difference for K^+ was rapidly decreased during the first 3 min after anoxia in control animals. Thereafter it decreased slowly and reached a minimum value of 24.8 mV 32 min after anoxia. The rate of decline of the K^+ electrochemical potential difference was slower in noise exposed guinea pigs and kanamycin treated guinea 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) around 30 min after anoxia. Thereafter, the rate of fall of $[K^+_{end}]$ began to increase and reached 500 μM/min 50 min after anoxia. In noise exposed guinea pigs the time course was similar to that observed in normal guinea pigs, but in kanamycin treated guinea pigs the rate of $[K^+_{end}]$ fall was markedly reduced.

The mean G_K averaged from 10 to 30 min after onset of anoxia was (34.85 ± 5.60) x 10^{-6} ohm^{-1} in normal guinea pigs, whereas the mean G_K averaged during the same period was (20.43 ± 2.53) x 10^{-6} ohm^{-1} in noise exposed guinea pigs and (8.13 ± 1.82) x 10^{-6} ohm^{-1} 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}$$cm^3sec^{-1}$ in noise exposed guinea pigs and (42.0 ± 19.08) x $10^{-9}$$cm^3sec^{-1}$ in kanamycin treated guinea pigs. These values were considerably lower than that of (193.62 ± 34.91) x 10^{-9} cm^3sec^{-1} observed in control guinea pigs.

The mean values of G_K and P_K in normal and noise exposed guinea pigs were found to be comparable with those computed from the rate constant for K^+, which was determined by the amount of uptake of ^{43}K into the endolymph.

The data were analyzed and a manuscript has been accepted to Exp. Brain Research for publication.

We plan to determine the possible correlation between the suppression of the cochlear microphonics and permeability changes in noise exposed animals by utilizing three compartment analysis. Some of the proposed course will be found in Project title "The effects of noise and drugs on the electrochemistry of the cochlea."

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is impossible at present to measure the permeability of the various cochlear membrane and one useful approach is to determine the diffusional alterations in the endolymphatic ionic concentration during anoxia. The present study will make a significant contribution to the understanding of noise induced ear damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50042-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	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)

Although the physiological effects of continuous noise on cochlear function are well documented in the literature, the effects arising from impact noise exposure have not yet been characterized. This is a direct consequence of the difficulty in generating and calibrating impact noise under laboratory conditions. A prototype impact noise generator has been used to test the widely accepted hypothesis that different types of noise of equal energy produce similar damage in the cochlea. Our results indicate that during 20 mins exposure and 1 hour recovery the suppression of responses produced by impact or continuous noise of equal energy are markedly different. The study of whether this difference persists after longer exposure and recovery periods awaits the development of a more durable noise generator which is presently under way.

PROJECT DESCRIPTION

METHODS EMPLOYED: Impact noise with peak sound pressures up to 132 dB sound pressure level (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 loudspeaker system. Conscious guinea pigs with implanted round window electrodes, or anesthetized guinea pigs with differential electrodes placed in the perilymphatic scalae, were exposed to a 20-minute period of impact noise or a similar period of broad band noise of equal energy. The suppression of tone induced responses (cochleae 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 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. Broad band continuous noise at 105 dB SPL had an equivalent energy content to this level of impact noise. 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 responses were 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 of this energy level. Impact noise produced a greater suppression of the CM and a smaller suppression of the AP than did continuous noise of equal energy.

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. However, it is not possible to extrapolate these data to give an indication of whether the differences persist in the long-term since our results indicate that recovery may follow different time courses for the two types of noise.

The project will, therefore, proceed to develop a more durable impact noise generator. This device will be used to expose guinea pigs for up to 8 hours a day for a period of several days. Cochlear responses will be monitored with implanted round window electrodes throughout the exposure and for several weeks afterwards. These experiments will provide valuable information concerning the potential hazard of impact noise under conditions similar to those experienced by workers in the industrial environment.

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 relationships between hearing loss and the parameters of impact noise exposure are essential for the development of adequate noise regulations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50043-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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. 10-76)

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.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50044-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	Peter Nawrot	Visiting Associate	LEB NIEHS

COOPERATING UNITS (if any)
Research Triangle Institute
Dept. of Nutrition, School of Public Health, University of North Carolina

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	.8	.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)

Significant adverse effects on the course of embryo development have been reported in several mammalian species after exposure to high levels of "noise" during pregnancy in some, but not all studies conducted. An investigation was conducted to determine whether the characteristics of different noise exposure paradigms contributed significantly to this variability. Accordingly,three differing paradigms, thought to be representative of a wide range of noises, were devised to simulate (1) semi-continuous exposure to extremely high levels (126 dBA jet engine noise), (2) unanticipated high intensity starting sounds (microprocessor controlled pseudorandom onset, fast rise time alarm bells, sonalert type warning devices, or jet engine noise each at 112 dBA), (3) very high frequency sound to compensate for the upward frequency shift in rodents' hearing relative to humans (18-20 kHz modulated times at 110 dB from a commerical rodent repelling device). Pre- and post-implantation exposure to the first two paradigms resulted in decreased pregnancy maintenance and embryo lethality. Postimplantation exposure resulted in significantly increased fetolethality. No exposure related changes in plasma corticosterone levels were found.

PROJECT DESCRIPTION

METHODS EMPLOYED: Mated female mice were exposed to noise in an Industrial Acoustics Corporation (IAC) double-walled audiometric room (4 x 3.5 x 3 m). The inside walls were lined with masonite for acoustical hardening purposes. The same chamber was also used for the quiet-environment control group. Ambient noise levels in the chamber before introduction of animals was less than 20 dB(A), as measured by a Bruel and Kjaer (B & K) type 2203 sound level meter. Because of noise generated by the animals themselves, Kimmel, et al. found that noise levels measured inside cages under similar conditions always exceeded 20 dBA. The housing conditions (temperature, humidity and light-cycle) were the same as provided in the animal quarters.

Sound amplification, control, and monitoring equipment were located outside the chamber; speakers and other sound producing equipment were suspended from a grid approximately one meter above the cages, which were located on the chamber floor.

Three noise exposure paradigms were used in this study. In the first experiment, sound was reproduced from a tape recording of extremely high intensity continuous jet engine noise obtained from the Aeromedical Research Laboratories, Wright Patterson AFB, Ohio. Although the levels produced by jet engine runup may exceed 140 dBA, this noise was reproduced at 127 dB(A), the highest level obtainable with the existing amplifier/speaker system. Cassette loop tapes were constructed from the master tape and each loop discarded after 12 hours.

To simulate conditions of random onset startling noise, an exposure paradigm involving microprocessor controlled pseudorandom presentation of three different sounds was employed in a second experiment. The three sounds were: (1) alarm bells at 110 dB(A). (2) a commercially marketed warning device called Sonalert, at 103 dB(A), and (3) a section of jet engine noise identical to that described previously, but reproduced at 110 dB(A). In order to insure that the animals would be exposed for at least a minimum period, the microprocessor was programmed to choose from one of 8 "on" periods (1,2,3,4, 5,6,7, or 8 minutes) then from one of 8 "off" periods (9,11,13,15,17,19,21, or 45 minutes).

High frequency sound from a device commercially marketed for repelling feral rodents was used in the third exposure experiment. Sound from this device, called Rodo Gard^{R}, was presented at 113 dB(Linear). Rodo Gard^{R} did not produce the entire 18-20 kHz spectrum simultaneously, but swept downward from 20 to 18 kHz over a 3-second interval, then repeated the process after a 100 msec delay.

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.

To investigate corticosterone levels in plasma of noise-exposed female mice, blood samples were obtained on days 1, 3 and 6 from experimental groups exposed to noise during days 1 through 6 of gestation. Blood was collected on days 6, 10 and 15 of pregnancy from experimental groups exposed during days 6 through 15 of gestation. Blood was obtained on days 1, 3, 6 and 15 of pregnancy from the control groups (animal quarters, chamber). At the onset at least 6 females from each experimental group were assigned randomly for blood collection on each designated day. To minimize the effects of diurnal variation in plasma corticosterone for all experimental groups, blood samples were always taken between 3:00 p.m. and 4:00 p.m. The animals were anesthetized by intraperitoneal injection of 8 ml/kg Nembutal and the blood samples taken from subclavian arteries directly into heparinized collect tubes by insiding the axillary region. To minimize any stressful effect on plasma corticosterone levels due to handling, blood collection was completed in less than 2 minutes.

Plasma corticosterone levels were measured by radioimmunoassay technique available from Endocrine Sciences (Tarzana, California) using rabbit antiserum obtained from Dr. G. Niswander (Fort Collins, Colorado) and radioactive 1,2#H-corticosterone (New England Nuclear Corporation). Assay for all samples were performed in duplicate and corticosterone values are expressed as mg/100 ml of plasma.

All experimental data were analyzed by nonparametric test procedures. The litter was considered as the experimental unit. Experimental groups were compared with controls by Mann-Whitney U test and Chi-square test. A $P \leq .05$ probability level was accepted.

MAJOR FINDINGS AND PROPOSED COURSE: Significant ($P < .05$) decreases in pregnancy rate were noted in all groups exposed to all noises except the group exposed to very high frequency noise post-implantation. Significant ($P < .05$) decreases in maternal and fetal weight gain occurred in the group exposed pre-implantation to high intensity startling sounds. Significant ($P < .05$) embryolethal effects occurred in the group exposed pre-implantation to the extremely high intensity jet noise paradigm, and significant fetolethal effects were associated with post-implantation exposure to the very high frequency noise paradigm. No significant teratogenic effects or corticosterone alterations were noted.

Because the increase in fetolethality resulting from post-implantation exposure to the high frequency sound was both dramatic and unique, it appeared to provide a model from which hormonal/biochemical alterations might be determined. Since the introduction of exogenous catacholamines has been reported to induce date stage fetolethality, the exposure was replicated and catecholamine levels measured (See 50045). This project is complete.

SIGNIFICANCE 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: Embryotoxicity of various noise stimuli in the mouse. P. Nawrot, R.O. Cook and R. Staples Teratology (Sept. 1980). Project completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50045-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 ES 50044) and to be unaffected by noise exposure, this experiment focused on catecholamines, because exogenous introduction of these substances has been found to increase fetolethality. Exposure period was 12 hours (noon to midnight).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Separate groups of pregnant mice were exposed to pre- and post-implantation high frequency noise (18-20 kHz) at 110 dB sound pressure level and their concepti examined for teratogenic and embryo-fetotoxic effects using standard techniques.

In addition, plasma catecholamines (epinepherine, norepinepherine, dopamine) will be assayed by a radioenzymatic-paper chromatographic technique described by Weise and Koper. A contract was let to the Biological Services Research Center, UNC School of Medicine, for this assay.

MAJOR FINDINGS AND PROPOSED COURSE: In previous experiments exposure of CF-1 mice to noise located in the most sensitive portion of their audibility curve resulted in a significant increase in fetolethality. This effect occurred around 20 KHz. The purpose of this experiment was to reconfirm the previous data and to determine the biochemical correlates of this result. The contractor has not finished the catecholamine assay at this point. Our earlier findings of noise induced dramatic late stage fetolethality increases was not confirmed. Late state fetolethality was increased, but the level of significance was much lower.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of hormonal/biochemical correlates of nonauditory effects of noise is a necessary step to understanding these effects sufficiently for predictive purposes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50046-02 LEB

PERIOD COVERED
January 1, 1979 to September 30, 1980

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:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
	R.P. Mason	Research Chemist	LEB	NIEHS
	A. Motten	NRS Postdoctoratoral Fellow	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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: $4\text{-}H_2NO_2SC_6H_5$, SO_2NH_2 $4\text{-}NH_2C_6H_5SO_2$, SO_3. Under the same conditions 4-aminobenzoic acid Yielded $4\text{-}HOOCC_6H_5$ and $H^{\cdot}$. Musk ambrette (4-t-butyl-3-methoxy-2, 6-dinitrotoluene) gave rise to a mixture of two nitroanion free radicals upon irradiation in methanol. The generation of these free radical species may explain the phototoxic and photoallergic properties of these compounds.

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 popular, 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.

MAJOR FINDINGS AND PROPOSED COURSE: In aqueous solution, sulfanilamide (4-aminobenzenesulfonamide) is photolyzed according to the following reaction scheme:

$$4\text{-}NH_2C_6H_5SO_2NH_2 \xrightarrow{h\nu} NH_2 + C_6H_5SO_2NH_2$$

$$\xrightarrow{h\nu} 4\text{-}NH_2\dot{C}_6H_5 + \dot{S}O_2NH_2$$

$$\xrightarrow{h\nu} 4\text{-}NH_2C_6H_5\dot{S}O_2 + \dot{N}H_2$$

The primary radicals produced also react further to give other radical and non-radical species.

$$2\ \ 4\text{-}NH_2C_6H_5\dot{S}O_2 \longrightarrow 4\text{-}NH_2C_6H_5SO_3$$

$$+NH_2C_6H_5SO_2^-$$

$$4\text{-}NH_2X_6H_5SO_3^- \xrightarrow{h\nu} 4\text{-}NH_2\dot{C}_6H_5 + SO_3^{\cdot -}$$

$$2\ \dot{S}O_3^- \longrightarrow SO_3 + SO_3^{2-}$$

$$SO_3 + H_2O \longrightarrow 2H^+ + SO_4^{2-}$$

Under the same conditions, 4-aminobenzoic acid was found to undergo the following reactions:

$$4\text{-}H_2NC_6H_5COOH \xrightarrow{h\nu} \dot{N}H_2 + C_6H_5\dot{C}OOH$$

$$\xrightarrow{h\nu} H^{\cdot} + H\dot{N}\,C_6H_5COOH$$

Other compounds which have also been studied include sulfacetamide, tolbutamide, carbutamide, chlorthiazide, hydrochlorthiazide and musk ambrette (4-t-butyl-3-methoxy-2, 6-dinitrotoluene). While these photosensitizers also give rise to free radical intermediates upon irradiation their structures have not yet been determined.

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. Photochem. Photobiol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50047-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	Donald D. Feldman	Veterinarian	CMB	NIEHS
OTHER:	Hugh Tilson	Research Pharmacologist	LBNT	NIEHS

COOPERATING UNITS (if any)
Comparative Medicine Branch, RRP, NIEHS
Laboratory of Behavioral and Neurological Toxicity

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	0.1	0.3

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 biological effects. The effect of three different fluorescent lighting systems (simulated daylight, coolwhite, pink) on the C_3H mouse are being studied. The parameters measured include longevity, tumor incidence, reproduction (litter size, sex ratio etc.), pathology and behavior. Results to date indicate that 50% of the bred females survived for 46 weeks, 50 weeks and 56 weeks in the daylight, pink and cool white exposed groups, respectively. Data on reproduction and pathology are still being evaluated.

PHS-6040
(Rev. 10-76)

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: This study has now been ongoing for 62 weeks. At the present time, the percent mentality of the female mice is 63, 72 and 80 for the daylight, pink and cool white groups, respectively. The corresponding fifty percent survival rates were 46 weeks, 50 weeks and 56 weeks. Data on reproduction (litter size, sex ratio, weaning weight) and pathology are still being evaluated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50048-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LEB	NIEHS
	Gary E.R. Hook	Senior Staff Fellow	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. 10-76)

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 Synva 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[5] 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 to thereby eventually duplicate the natural fluidity profile and thereby to 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 intercalated 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. Little was accomplished during the past year on this project, so we are continuing the project for another year.

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 branchiotres. 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50049-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 anti-cancer 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. 10-76)

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 melatin 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 anticancer drugs. Biochem. Biophys. Acta. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50050-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectra of anaerobic microsomal incubations at 25° were obtained with a Varian century series E-109 spectrometer equipped with a TM_{110} cavity. Visible spectra of *cis*- and *trans*-furylfuramide (AF-2) were obtained with a DW-2A Aminco-Chance spectrophotometer at 37°.

MAJOR FINDINGS AND PROPOSED COURSE: Tatsumi *et al.* have proposed that enzymatic *cis-trans* isomerization of furylfuramide is a direct consequence of enzymatic nitro reduction. The nitroreductases, which are inhibited by oxygen, transfer a single electron to nitro substrates to give their respective anion free radicals. The carbon-carbon double bond linking the two furan rings of AF-2 would be weakened by anion radical formation, because the additional electron is in an antibonding molecular orbital. Upon formation, the *cis*-AF-2 anion free radical was proposed to isomerize rapidly to the *trans*-AF-2 anion, which could then be oxidized to form *trans*-AF-2.

Recently, Tomoeda and Kitamura studied the isomerization and nitro reduction of *cis*-AF-2 by subcellular fractions of *E. coli*. Their observations suggested that the nitro reducing and isomerizing activities of *E. coli* were due to totally different enzymes, in apparent contradiction of the isomerization mechanism of Tatsumi *et al.*

The ESR spectrum of an anaerobic microsomal incubation containing furylfuramide and an NADPH-generating system provides direct evidence of free radical formation. In the presence of air, nitroaromatic anion free radicals undergo rapid air oxidation to form superoxide anion, and the nitroaromatic anion free radicals not detected. In addition, identical spectra were obtained with either *cis*- or *trans*-AF-2. These ESR spectra did not vary with time even though visible spectroscopy shows a rapid and nearly complete conversion of *cis*-AF-2 to *trans*-AF-2.

Although spectral evidence alone indicates that both the major and the minor components of the spectrum represent AF-2 anion free radicals, the assignment of the spectrum to *cis-trans* anion free radicals must be made on other grounds. INDO (intermediate neglect of differential overlap) molecular orbital calculations on *cis*- and *trans*-AF-2 anions suggest that the *cis* anion radical will have the larger nitrogen hyperfine splitting constant. Furthermore, the greater steric hindrance expected for the *cis*-conformer should result in a predominance of the *trans*-conformer. In the parent compounds, the greater thermodynamic stability of *trans*-AF-2 (87-91%) vs that of *trans*-AF-2 to *cis*-AF-2 (7-11%) observed in xanthine oxidase incubations under optimum conditions. Clearly, since xanthine oxidase is only a catalyst, and cannot change the thermodynamic equilibrium of *cis*- *trans*-AF-2, the equilibrium ratio of *cis*- to *trans*-AF-2 and the two free radicals is the strongest chemical evidence for our assignment of the two species as the respective anion radicals. The observation of distinct conformational isomers with ESR only requires that the equilibrium mixture be slowly interconverting on the

ESR time scale, which is very rapid. More precisely, the lifetime of the conformers must be much longer than $1/\gamma_e (a^N_{cis} - a^N_{trans}) = 28$ nanoseconds.

The reaction can still be fast in terms of chemical reaction, and in fact, competes favorably with the autoxidation of the AF-2 anion free radicals.

The results of Tomoeda and Kitamura and the isomerization mechanism of Tatsumi *et al.* are not contradictory if the oxygen-insensitive *E. coli* reducing activity does not form the anion radical intermediate and the oxygen-sensitive *E. coli* reducing activity does form the anion radical, as has been recently demonstrated. In the present study, we provide electron spin resonance evidence for *cis*- and *trans*-AF-2 anion radical intermediate formation during the microsomal *cis-trans* isomerization of AF-2.

Higher resolution ESR investigations in D_2O buffer, in conjunction with INDO calculations, support this concept by unambiguously demonstrating the enzymatic generation of *cis* and *trans* radical anions of 3-(5-nitro-2-furyl)-2(2-furyl) acrylamide. The INDO calculations further indicate that the rotational barrier between the *cis* and *trans* anion radicals of this compound is only 5-10 kcal/ mole, whereas a 70 kcal/mole barrier exists for the parent geometric isomers. Hyperfine splitting constants for the *cis-trans* conformers have been assigned on the basis of INDO calcualtions. 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

Mason, R.P., Kalyanaraman, B. and Perez-Reyes, E.: Electron spin resonance evidence for a free radical intermediate in the cis-trans isomerization of furylfuramide by oxygen-sensitive nitroreductases. Mol. Pharm. 16: 1059-1064, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50051-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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. 10-76)

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 P-450 and b_5 and their respective flavin-containing reductases, NADPH-cytochrome P-450[5] and NADPH-cytochrome b_5 reductase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tetracyano-compounds 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50052-02 LEB

PERIOD COVERED

October 1, 1979 to September 30, 1980

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

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Chemical reduction of the highly active quinone-containing antitumor drugs, adriamycin and daunorubicin formed the same partially reduced free radical previously reported by microsomal activation. *In vitro* incubation of the chemically activated free radical intermediates with DNA resulted in covalent binding of these drugs to DNA. The adriamycin semiquinone radical has a greater affinity for DNA and covalent complexes containing up to one adriamycin per 15 nucleotides were obtained. The daunorubicin semiquinone radical, on the other hand, showed a lesser binding affinity and gave rise to complexes in which one drug molecule was covalently bound per 140 nucleotides. Studies with synthetic polynucleotides suggest that these drugs have a high preference for poly (dG) and poly (dC). Microsomal activated drugs also bind covalently to DNA with identical binding affinities. Mg^{+2} decreases this binding. Longer incubation also decreases binding.

PHS-6040
(Rev. 10-76)

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. We had also shown that adriamycin had a greater binding affinity for DNA than daunorubicin. Furthermore, we had also shown that this binding affinity in vitro correlates with their ability to indule sister chromatid exchanges.

MAJOR FINDINGS AND PROPOSED COURSE: Recently, we have examined the reactions of activated drugs to RNA and denatured DNA. Our results show that the denatured DNA is a better substrate for this binding than the native DNA. On the other hand, RNA serves as a poor substrate and the binding is considerably lower.

Binding of adriamycin and daunorubicin decreases with increasing ionic strength. This is probably due to decreased noncovalent binding of the drugs at high salt concentrations and covalent binding proceeds such intercalative binding. The time course of covalent binding between activated drugs and DNA indicate that reactions are essentially complete in 30 min.

In order to investigate the binding sites in nucleic acids, binding studies with synthetic polynucleotides were carried out under identical conditions. Reductively activated adriamycin and daunorubicin show a high preference for guanine containing polymers. In addition, poly(dC) is also an excellent substrate and shows high binding. Polymers containing either poly(dA) or poly(dT) are not as good substrates as those containing either poly(dG) or poly(dC). Again, adriamycin, shows a much greater affinity than daunorubicin, except with polymers containing guanine and cytosine.

Sato et al. and Bachur et al. have shown that incubation of adriamycin and danorubicin with microsomes in the presence of NADPH results in the formation of the semiquinone metabolites. We have investigated whether enzymatically activated drugs will bind covalently to DNA. Incubation of enzymatically activated (microsomes -NADPH regenerating systems) adriamycin and daunorubicin with DNA resulted in covalent binding. Our results show that a longer incubation time decreases binding. During the isolation of drug-DNA complexes, it was noted that large amounts of the drugs were bound to the microsomes which could not be extracted by ethanol or NaCl. Apparently, the drugs also bind covalently to the microsomal proteins.

Microsomal catalyzed C_7- reductive cleavage of adriamycin and daunorubicin is well known. Recently, Mason has suggested that the semiquinone may be an intermediate in the formation of the deoxyaglycone products. This mechanism proposed the formation of a carbon centered (C_7-) radical from the semiquinone free radical. The apparent dimerization products linked at the C_7- position have been isolated from three different anthracyclines (Aclacinomycin A, Alkavinone and 1-Deoxypyrromycin), lending support to the formation

of the C_7-radical. Recently, Moore has proposed a mechanism for the bio-activation of anthracycline antitumor drugs into covalent binding species. In this postulation, a quinone methide (with carbocaion character at C_7-) is presumed to be an active alkylating agent. In addition to alkylation of nucleic acids and proteins, this "active species" could lead to the formation of deoxyaglycone and dimerization products through a H-transfer and a Michael addition.

The formation of either the C_7- radical or the C_7-quinone methide from adriamycin and daunorubicin is very interesting and could explain why there is a decrease in binding upon a longer incubation period prior to addition of DNA from microsomal NADPH catalyzed reactions. The concentration of the "active species" is time dependent due to two independent but simultaneous reactions: a) abstraction or transfer of a proton to form deoxyaglycone products and b) dimerization products. These competing reactions will effectively reduce the availability of the "active species" for alkylation of nucleic acids with time and hence reduce binding.

Future Plans include characterization of the adduct by enzymatic degradation and to explore and differentiate between C_7-free radical or C_7-quinone methide ediated alkylation of nucleic acids.

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 Chignell, C.F. Binding mode of chemically activated semiquinone free radicals from quinone anticancer agents to DNA. Chem. Biol. Interact. 28:301-308, 1979.

Sinha, B.K. Binding specificity of chemically and enzymatically activated anthracydine antitumor agents to nucleic acids. Chem. Biol. Interact. 30:67-77, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50053-02

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The cytotoxic and mutagenic properties of antitumor drugs such as adriamycin, acridines, diacridine, actinomycin D and Pt compounds are related to their interaction with nucleic acids and inhibition of protein systhesis. We have examined their interaction with human erythrocyte ghost membranes and murine mastocytoma cells using spin labeling techniques. These drugs induce changes in electron spin resonance of the spin labeled ghost membranes and in the mastocytoma cells. These findings suggest that these drugs induce changes in protein conformation of the membranes. The membrane binding properties of these drugs may be important in their mechanism of action.

PHS-6040
(Rev. 10-78)

PROJECT DESCRIPTION

METHOD EMPLOYED: In previous studies we have shown that a number of antitumor drugs (which bind to nucleic acids 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. On the other hand, incubation of the drugs with mastocytoma cells produced two distinct binding sites as indicated by fluorescence. In addition to the bright yellow membrane fluorescence seen with ghosts, red fluorescence localized in the nucleus was also observed in some of these cells. Bachur, et al. and Krishan et al. have shown that these drugs bind to nucleic acids and appear as red orange fluorescence. Further characterization of these cells using trypan blue exclusion indicated that cells with orange-red nuclear fluorescence are not viable cells. These findings suggest that, at low concentrations, the drugs primarily bind to the membranes and that binding to nucleic acids is only observed following cell death.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND 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.

PUBLICATION

Rakhit, A. and Chignell, C.F.: A spin label study of horseradish peroxidases Biochem. Biophys. Acta, 580: 108-119, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50054-02 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

CHECK 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 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. Ingal et al. using the spin trap 2-methyl-2-nitrosopropane (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 nonfice radical reaction of MNP with the microsomal lipids. Studies using PBN and $C^{13}Cl^4$ show no indication of a ^{13}C hyperfine splitting in either UV photolysis experimenter or microsomal incubations further precluding the trapping of the trichloromethyl radical.

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 all of 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

Kalyanaraman, B., Mason, R.P., Chignell, C.F., Perez-Reyes, E., Wolf, C.R. and Philpot, R.M.: Characterization of the free radical formed in aerobic microsomal incubations containing carbon tetrachloride and NADPH. Biochem. Biophys. Res. Comm. 89: 1065-1072, 1979.

Wolf, C.R. Harrelson, W.G., Jr., Nastainczyk, W.M., Kalyanaraman, B. and Mason, R.P.: Reductive metabolism of carbon tetrachloride and lipid peroxidation. Mol. Pharm. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50055-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

OTHERS: None

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

METHODS EMPLOYED: Streptonigrin (SN, NSC 45383), an antitumor antibiotic containing the aminoquinone moiety inhibits the growth of certain tumors, e.g., carcinoma 755 and Sarcoma 180. SN also inhibits DNA and RNA synthesis in tissue culture cells and brings about degradation of DNA when administered to bacterial cells. Although the biochemical mechanism of SN has remained poorly understood, White et al. have shown that the bactericidal action of SN requires both intracellular reduction of SN and the presence of O_2. The antibiotic has also been shown to generate superoxide ($O_2^{\cdot}$-) in the degradation.

Chemically activated Streptonigrin (with or without $Zncl_2$, $Mgcl_2$) was incubated with nucleic acids under N_2 atmosphere for 18 hrs at room temperature.

The stable SN- nucleic acids complexes were obtained by dissolving them in 6 M NaCl, stirring for 1 hr and precipitating with ethanol. The dissociated drugs were removed; then the complexes were isolated and washed with ethanol. The complexes were dried, dissolved in phosphate buffer and extensively dialyzed against 10 mM phosphate buffer containing 1 M NaCl and chromatographed on Sephadex G-100 using 10 mM phosphate containing 50 mM NaCl as eluant.

Hepatic microsomes were prepared from male C-D rats by homogenizing livers in 150 mM KCl- 50 mM Tris buffer at pH 7.4 according to the procedures of Mason et al. Microsomal activation of SN was carried out at room temperature by dissolving SN with or without metals. Glucose-6-phosphate (1.5 mg/ml), glucose-6-phosphate dehydrogenase (5 units), and microsomal protein (2 mg/ml) were added to the drug solutions. The reaction was started by adding NADPH (0.5 mg/ml) and after 5 min deaerated DNA solution. The mixtures were incubated at room temperature in the dark for 2.0 hrs and the SN-DNA complexes were isolated by centrifugation at 20,000 g for 15 min. The supernatant was removed and added to large volumes of ethanol. The SN-DNA complexes from the ethanol precipitate were isolated as before.

MAJOR FINDING AND PROPOSED COURSE: Incubation of SN in vitro with DNA, in the presence of Zn^{2+}, resulted in irreversible binding of SN to DNA, and complexes containing 1 SN adduct per 400 nucleotides were obtained. However, in the presence of a reducing agent ($NaBH_4$) and Zn^{2+}, considerably more SN was bound to DNA, and stable complexes containing 1 SN adduct per 80 nucleotides were obtained. Furthermore, chemically activated SN, in the absence of Zn^{2+}, showed a lesser binding affinity for DNA, and 1 SN adduct per 250 nucleotides were obtained. The presence of Mg^{2+} in the incubation mixture also decreased this binding. When denatured DNA was used as a substrate, more SN was bound. The increased binding of SN to denatured DNA idicates that its binding is facilitated by the loss of the double helical structure.

In order to evaluate the role or reduction on binding, we have carried out these reactions under different reducing conditions. When performed SN-DNA complexes were activated with $NaBH_4$, more SN was bound to DNA. These observations suggest that the "active intermediate(s)" which binds to nucleic acids

is probably unstable and decays through some side reactions. Similarly, increased binding was observed when the antibiotic was completely reduced, presumably to the hydroquinone, by $NaBH_4$. These data suggest that the "active intermediate(s)" which binds to nucleic acids is probably derived from the fully reduced SN. This is further substantiated by the observation that maximum binding was obtained only when performed SN-DNA Complexes were activated by adding two equivalents of $NaBH_4$ in a single step.

In order to evaluate the nature of the binding sites in nucleic acids, studies were carried out with various single-stranded and double-stranded synthetic polynucleotides under identical conditions in the presence of Zn^{2+}. The results presented in Table 3 show that while SN has a high affinity for guanine, cytosine and adenine also serve as excellent substrates for its binding. Secondary structure retards the preferential binding to guanine since twice as much SN was bound to polydA-polydT than to polydG-polydC.

Incubation of enzymatically activated (microsomal proteins-NADPH regenerating systems) SN with DNA also resulted in irreversible binding. While the presence of Zn^{2+} had no effect on this binding, Mg^{2+} did decrease binding slightly. It is noteworthy that microsomally activated SN binds more extensively to DNA than the chemically activated SN. While the reason for this phenomenon is not clear, identical binding with or without Zn^{2+} may be caused by the presence of Zn^{2+} may be caused by the presence of Zn^{2+} in the microsomal proteins.

The present study indicates that, in the presence of reducing agents, SN binds to nucleic acids. This binding of SN is stable under the conditions known to dissociate physically bound drugs, such as (a) extensive dialysis against 1 M NaCl; (b) 6 M NaCl, 1 hr; and (c) sephedex G-100 chromatography indicating that SN is irreversibly bound to nucleic acids. Furthermore, the present study indicates that SN-Zn^{2+} complexes in the absence of reducing agents also bind to DNA. However, this binding of SN to nucleic acids is greatly enhanced by ZN^{2+} and $NaBH_4$ suggesting that this metal ion may play an important role in the biological action of SN.

The nature of the "active intermediate(s)" which alkylates nucleic acids is not known at this time. However, these studies indicate that this intermediate is derived from the fully reduced SN. If the partially reduced intermediate (semiquinone radical form) were the active species, then one might anticipate increased binding when the drug is reduced by small portions of the reducing agent, as has been reported for mitomycin C and the anthracyclines. Such an increase in binding was not observed. The results are consistent with the earlier observation of White et al. that SN causes a decrease in the viscosity of DNA only in the presence of large excess of $NaBH_4$. This study further indicates that the primary reduction products are unstable and that they decompose, since higher binding was obtained when SN was activated in the presence of DNA rather than exposing DNA to the reduced drug. LaRusso et al. have obtained similar results with reductively "activated" metronidazole.

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 An^{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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50056-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	RRP	Senior Scientist

COOPERATING UNITS (if any)

Research Resources Program

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:
0.7	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)

It has been documented that the cochlear potentials are maintained by metabolic energy. However, there is little conclusive evidence whether a decrease of metabolic rate by hypothermia can alter ion movement or membrane permeability of the cochlea. The present study is designed to determine dependence of cochlear membrane permeability on temperature.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Healthy guinea pigs anesthetized with sodium pentobarbital were used. The sound induced responses were recorded with differential electrodes placed in the basal turn of the cochlea. The rectal temperature and blood pressure in common carotid artery were monitored. The body 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 a steady state of hypothermia. Total concentrations of K^+ and Na^+ in the cochlear fluids were determined by a helium glow photometer and radio activities of ^{43}K and ^{22}Na were determined by a gamma spectrometry system.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Cochlear temperature, heart rate and blood pressure. The temperature of the perilymph of the scala tympani was 34.5° ± 0.7°C under open bulla condition when the rectal temperature was kept at 39.0° ± 0.5°C. The difference between the cochlear and rectal temperature gradually decreased as the body temperature was lowered. At a rectal temperature of 29.0° ± 0.5°C, the cochlear temperature was 26.2° ± 0.6°C. In animals which were immobilized with gallamine triethiodide and artifically respirated, the arterial blood pressure was increased and the heart rate decreased during hypothermia.

2. Cochlear potentials. The EP measured at 39°C was 86.0 ± 3.5 mV. When the rectal temperature decreased to 29.0°C the EP was 78.2 ± 3.6 mV. The EP recorded 2 hours after the rectal temperature was kept at 29.0° ± 0.5°C was 73.2 ± 3.9mV. During course of hypothermia the magnitude of cochlear micro-phonics (CM) gradually decreased. Usually a large increase of the negative summating potential accompanied the decrease in CM during the period of hypothermia. The action potentials (AP) in response to test stimuli of low intensity were markedly suppressed, but the input-output function of the AP remained little changed or became steeper with intermediate or high intensity stimuli.

3. Electrolyte concentrations in the cochlear fluids. Hypothermia did not result in marked changes in K^+ concentrations in the endo- and perilymph of non-perfused cochlea. The Na^+ concentrations in both endolymph and perilymph were slightly decreased in hypothermic guinea pigs.

	$[K^+]$ in mM/L	
	Hypothermia	Control
Perilymph (SV)	6.1 ± 0.5	7.3 ± 1.1
Perilymph (ST)	3.7 ± 2.0	3.5 ± 0.3
Endolymph	156.2 ± 8.3	155.9 ± 7.6
n	5	6

$[Na^+]$ in mM/L

	Hypothermia	Control
Perilymph (SV)	147.4 ± 13.2	154.4 ± 5.7
Perilymph (ST)	145.0 ± 16.1	155.4 ± 1.1
Endolymph	0.6 ± 0.5	1.5 ± 0.2
n	6	5

4. Distribution of ^{43}K and ^{22}Na in the cochlear fluids. 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 as a function of a duration of perfusion in both control and hypothermic guinea pigs. The rate constant for K^+ and half-time of exchange were 0.0069 min^{-1} and 100.5 min respectively in hypothermic guinea pigs, which were significantly lower than the respective values obtained in normal guinea pigs (rate constant 0.013 min^{-1} and half-time 55.0 min).

The K^+ conductance of the endolymph-perilymph barrier can be computed by using the following equation

$$G_K = \frac{\lambda_K F \, V_{end}[K^+ end]}{\Delta\tilde{\mu}_K / F}$$

where V_{end}, volume of the endolymph (2 μl)

$\Delta\tilde{\mu}_K$ electrochemical potential difference for K^+ between endolymph and perilymph

The computed G_K was 20.65 x 10^{-6} ohm^{-1} in hypothermic guinea pigs which was considerably lower than 35.16 x 10^{-6} ohm^{-1} in normal guinea pigs. The experimental data were analyzed and a manuscript is in preparation and will be submitted for publication in the near future.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Dependence of the cochlear potentials on metabolic energy has been emphasized in estimating ear damage by noise or drugs. However, little information is available concerning changes in ion movement and membrane permeability in the cochlea produced by decrease of metabolic rate. 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 suppress not only the active ion transport, but also the membrane permeability of the cochlear partition.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50057-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	M. J. Galvin	Senior Staff Fellow	LEB	NIEHS
OTHERS:	D. I. McRee	Research Physicist	LEB	NIEHS
	C. H. Hall	Graduate Student	N.C. State University	
	J. P. 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 Park, N.C.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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 3.5×10^8 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 1, 10 and 50 mW/g. The waveguide exposure system maintained the control (non-irradiated) and irradiated samples at 40 ± 0.15°C throughout the experiment. Before and after irradiation, aliquots of the suspensions were removed and the viabilities were determined. 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 the sperm into the suspending media was also determined. Controls released approximately 6 and 18% for LDH and GOT, respectively and the exposed had similar values. Thus, microwave radiation appeared to have no adverse effects on the parameters measured for mature spermatocytes irradiated in vitro. Other aspects of sperm physiology following microwave radiation are now being examined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: 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 mls 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 x 108 sperm/ml.

The sperms were irradiated in an S-band waveguide chamber at a frequency of 2450 MHz. The microwave generator was coupled to the waveguide using coaxial to waveguide adapters, and the wavelength was matched to air using a 1/4Y g matching dielectric. The waveguide was filled with distilled water which was continuously circulated to maintain temperature of the suspensions at 40.0+.1°C. Two Pyrex tubes were inserted into the waveguide, parallel to the E-field of the incoming radiation. While tube A received radiation, tube B, located 9.5 cm away from the dielectric plate did not, and served as the nonirradiated (control) sample. Each tube contained 4.2 mls 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.

The specific absorption rate (SAR) of the sperm suspension was calculated from time temperature profiles of irradiated samples measured by a Vitek noninteracting temperature probe. The sperm were irradiated at SAR's of 1, 10, and 50 mW/g for 30 minutes. For each irradiated sample, there was a matched nonirradiated sample. A nonstirred, nonincubated sample was obtained from the stock suspension and the viabilities were determined using an accepted staining technique. 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 the sperm into the suspending media was also determined.

MAJOR FINDINGS AND PROPOSED COURSE: Sperm viability was 96% initially, and was not affected by microwave radiation at any of the exposure levels examined. In addition, preliminary results indicate that microwave radiation has no effect on the permeability of sperm cells to the cytoplasmic enzymes, LDH and GOT. Transmission election micrographs revealed that there were no significant durational differences between non-irradiated and irradiated sperm cells. The data from these studies suggest that mature spermatocytes are unaffected by microwave radiation in vitro.

In addition to completing the above study, a second phase of the study involves artificial insemination of Nicholas white turkey hens. Forty-five hens will be divided into three groups of 15 each and receive semen from one of three treatment groups: non-irradiated semen, semen irradiated at 10 mW/g,

or semen irradiated at 50 mW/g. The sperm irradiation protocol will be the same as described in the methods, however, the seminal plasma will not be removed. Each hen will receive a single inseminiation of 0.4 ml semen (2.0×10^8 sperm). All hens will be individually caged in a single room. Eggs will be collected daily and incubated weekly. Egg production, fertility and hatchability will be determined for a 60-day period. In addition, the physiological condition of the young turkey plots will be determined at 4 weeks of age.

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

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50058-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	Peter Nawrot	Visiting Associate	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
OTHER:	Minuro Inouye	Visiting Fellow	LEB	NIEHS

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:
0.5	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)

Pregnant mice (CD-1 strain) were exposed to 2.45-GHz microwave radiation at power density levels of 5,21 and 30 mW/cm^2. The group exposed to 5 mW/cm^2 were irradiated for eight hours per day (4 hours exposure - 1 hour in home cages for food and water - 4 hours exposure) from day 1 to 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 temperature 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 ≃ 5.2 mW/g) and 21 mW/cm^2 (SAR ≃ 22.3 mW/g) did not produce any adverse maternal or embryofetal effects. At exposure to 30 mW/cm^2 (SAR ≃ 32 mW/g) during days 1-6 a significant decrease in implantation sites per litter and average fetal weight was observed. Exposure to 30 mW/cm^2 during days 6-15 resulted in a slight increase in the number of malformed fetuses (3.1 percent in the microwave exposed group, 1.7 percent in the 31°C elevated temperature group). The predominate malformation was cleft palate.

PHS-6040
(Rev. 10-76)

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: Our investigation showed that exposure of pregnant CD-1 mice to 2450-MHz CW microwave radiation for 8 hours per day to 5 mW/cm^2 (exposure during days 1-15 of pregnancy) and 21 mW/cm^2 (exposure of one group during days 1-6 and a second group days 6-15) did not produce any maternal or embryofetal changes in the parameters measured in this study. At exposure to 30 mW/cm^2 during days 1-6 a significant decrease in implantation sites per litter and average fetal weight was observed. Exposure to 30 mW/cm^2 during days 6-15 resulted in a slight increase in the number of malformed fetuses, primarily cleft palate.

The small, but statistically significant increase in malformed fetuses in the microwave exposed group (3.1 percent) in comparison to the corresponding elevated temperature group (1.7 percent) indicates that the threshold for induction of teratogenic effects is approximately 30 mW/cm^2 (SAR $\simeq$ 32 mW/g) for our experimental conditions.

We plan to repeat the experiment using the 30 mW/cm^2 exposure condition in order to verify the results reported above.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50059-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	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.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: 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.

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 AND PROPOSED COURSE: 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 mer-cuty vapor light source. In the intact erythrocytes, phototoxic lysis began between 6-8 min after the beginning 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. 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 plan to study 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 dichroism 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 effect of protoporphyrin on "inside out" and resealed ghosts will also be determined; in addition, since spectrin can be easily removed from the ghosts, CD studies of isolated spectrin and spectrin-depleted ghosts will be performed.

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.

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.

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.

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 symptom of phototoxic urticaria.

PUBLICATION

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50060-01 LEB

PERIOD COVERED
October 1, 1979 - September 30, 1980

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 Univ.	
	J. P. 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, NIH, Research Triangle Park, N.C. 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 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 developed which can maintain the specimens at physiologic temperatures (37°C ± 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. Other aspects of cell and organelles are currently being studied.

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 are exposed to SAR's of 1, 10, 50 and 100 mW/g for up to 90 minutes. The cells exposed include mast cells (see Z01 ES 50039-02 LEB) cardiac cells, peritoneal and alveolar macrophages, 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.

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 have been extended to include other aspects of lysosome physiology such as the response to drugs, and toxic compounds.

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. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50061-01 LEB

PERIOD COVERED
October 1, 1979 - September 30, 1980

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 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:
0.2	0.15	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)

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.

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 has been completed and there are no further experiments planned.

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, present 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. These studies 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

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50062-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Enzymatic Reduction of C-Nitroso Compounds

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

PI:	Ronald Mason	Research Chemist	LEB	NIEHS
	B. Kalyanaram	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.

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.

$$R\text{-}N = O + AH \quad R\text{-}\dot{N}\text{-}OH + A^{\cdot-}$$

$$R\text{-}\dot{N}\text{-}OH + O_2 \quad R\text{-}N = O + O_2^{\cdot-}$$

A nitroxide free radical is more likely to be the species formed as can be confirmed by ESR.

$$R\text{-}N = O + AH^- \quad R\text{-}\overset{O}{\overset{\cdot}{N}}\text{-}H + A^{\cdot-}$$

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.

$$AH^- + R\text{-}\overset{O.}{N}\text{-}H \longrightarrow AH^{\cdot} + R\text{-}\overset{H}{\overset{O}{N}}\text{-}H$$

$$R\text{-}\overset{H}{\overset{O}{N}}\text{-}H + O_2 \longrightarrow R\text{-}\overset{O.}{N}\text{-}H + O_2^{\cdot-}$$

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-Nitrosoquinoline-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 traps in chemical and biochemical systems. A cautionary note. Tetrahedron Letters 50, 1979, 4809-4812.

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: Prior to constructing a fluidized-bed photoreactor, preliminary experiments were conducted to determine optimal chamber dimension, gas flow rates for continuous circulation and irradiation of all particles, and particle mass load. The photoreactor which has been constructed is a glass, water-jacketed column, approximately 450 cm in length, 2.5 cm in diameter, with fritted discs at the base and top of the column which allow for the flow of gases. A particle mass-load of 0.5-1.0 g and gas flows of 10-20 liters per minute give a bed height of approximately 10 cm (length of lamp) for particles of densities 0.5-2.75 g/cm^3, 100-200 μm diameter.

MAJOR FINDINGS AND PROPOSED COURSE: The adsorption and extraction of pyrene have been investigated on glass, an inert substrate and on Carbosieve S, a carbon substrate. Glass beads can be evenly coated by evaporating solvent from a slurry of the glass beads and pyrene solution in a rotary evaporator, under vacuum. Carbosieve S readily adsorbs pyrene from solution (<99.9%). Sonication extration with methanol was found to be three times as efficient as with cyclohexane or dichloromethane. While extraction efficiency for methanol approaches 100% for pyrene adsorbed on glass, the extraction efficiency for Carbosieve S was only a few percent. Preliminary investigations indicate that carbon disulfide is a more efficient solvent for extraction of Carbosieve S.

As a high performance liquid chromatograph (HPLC) is now available, methods are being developed for the separation and analysis of the PAH compounds and their photolysis products. The more polar, oxidized hydrocarbons produced by photolysis of PAH can be more readily separated and analyzed by HPLC.

A photolysis experiment was conducted in the prototype photoreactor. Pyrene-coated glass particles were irradiated in dry air for 322 minutes at room temperature. Samples were withdrawn at various intervals, extracted and analyzed. The half life for the adsorbed pyrene (0.34 mg/g glass) was approximately 160 minutes and the photodegradation appeared to follow a first-order rate law with a rate constant of 4.5×10^{-3} minute $^{-1}$. No fluorescent products were observed in the extracts of two of the samples which were chromatographed on thin-layer plates. Separation and analysis of the extracts by high pressure liquid chromatograph indicated that six different products were formed during photolysis. One product has been tentatively identified as 1,8-pyrenedione.

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., M.A. Leyko and T.J. Kneip. Source identification and allocation of PAH Compounds in the New York City aerosol: methods and applications. In: *Carcinogenesis, A Comprehensive Survey, Vol. 4*, Eds., P.W. Jones and R.I. Freudenthal. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, p. 201, 1979.

Daisey, J.M. Organic compounds in urban aerosols. *Ann. N.Y. Acad. Sci.* In press.

UNIVERSITY OF MIAMI

TITLE: "Protracted Noise Exposure and Cardiovascular Function"

CONTRACTOR'S PROJECT DIRECTOR: Ernest A. Peterson, Ph.D.

PROJECT OFFICER (NIEHS): Teruzo Konishi, M.D., Ph.D., Visiting Scientist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: January 1977

CURRENT ANNUAL LEVEL: $40,000

PROJECT DESCRIPTION

OBJECTIVES: Because of the serious and pervasive nature of cardiovascular diseases, including hypertension, the relationship between noise and cardiovascular function has received particular attention. The objectives of this contract are to specify (1) the extent to which protracted noise exposure produces cardiovascular adjustments, (2) the dynamics of those adjustments, and (3) the nature and mechanisms of adaptation to noise. Semi-restrained rhesus monkeys are used for the work.

METHODS EMPLOYED: Rhesus monkeys will be exposed for a period of up to one year to noise conditions resembling those presently and potentially existing in the community and work place. Their cardiovascular responses will be compared to those of a group of rhesus monkeys maintained in relatively quiet conditions during the same period. Evidence for acoustic injuries and other organic pathology will be sought. During the course of the study, the general health condition will be monitored by various clinical tests in addition to appropriate physiological and biochemical tests relevant to cardiovascular function.

MAJOR FINDINGS AND PROPOSED COURSE: Two experimental monkeys were successfully exposed to term (8 months). Control data was acquired from serially exposed controls due to unexpected loss of parallel controls due to catheter failure. Blood pressure of experimental animals significantly exceeded that of controls and significantly exceeded experimental's baseline values two months post exposure (at sacrifice) indicating permanence of induced effects. The existence of data in the literature regarding the blood pressures of unrestrained rhesus monkeys also provides an indirect reference base. The blood pressures of the two experimental monkeys after six months of chair restraint but no noise exposure were at the 50th percentile with respect to the indirect reference base, but the diastolic pressures had risen to the 99th percentile after six months of noise exposure. The results appear to implicate stroke volume changes rather than increase in peripheral resistance as the source of the systolic rise, since pulse pressure has not decreased but risen. In the second phase of the experiment, stroke volume is being measured directly by

placing a flow probe on the aortic arch. Other physiological (dpdT, epicardial EKG and cardiac output) and biochemical (blood chemistry profile including SMA 40 and plasma catecholamine and cortisol level) parameters are being measured in the second phase of the project. EPA is the major funder of the project; NIEHS contribution has been principally in scientific monitoring.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The work will provide scientific information which is vital to further refine our concepts of how long-term exposure to contemporary noise levels affects the cardiovascular system in humans.

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 is 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: All instrumentation and techniques for measuring single-unit responses in the cochlea during exposure to microwave radiation has been developed and evaluated. Reliable detection of action potentials has been achieved by rejection of unwanted interference and by elimination of stimulus artifacts. Development of software for on-line analysis and display of neural data has been completed. Using this software, parameters for computer-controlled delivery of microwave and acoustic stimuli can be specified, immediate feedback from quantitative analysis of single-unit responses can be obtained, and appropriate changes in experimental execution to maximize the rate at which useful data are collected can be made. Responses of single fibers in the auditory nerve of cats are now being characterized using both acoustic and microwave stimuli.

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 should provide information on the nature of the receptors that are responsible for mediating the effects of microwave radiation on brain activity.

LABORATORY OF ENVIRONMENTAL CHEMISTRY

Environmental chemistry involves studies of the chemistry of compounds and their mixtures present in the environment which show undesirable biological or otherwise deleterious effects on living organisms with particular emphasis on xenobiotic, persistent molecules. Such environmental agents can include elements and both natural and synthetic organic and inorganic compounds. Environmental health chemistry attempts to bring such activities to a focus on potential human hazards of environmental agents, singly and in combination. The major interrelated divisions of environmental health sciences which are necessary to define a hazard are environmental epidemiology, toxicology and chemistry. The increasing importance of chemistry as a research base in these relationships has been recognized by the renaming of the chemistry programs at NIEHS to Laboratory of Environmental Chemistry. The lipophilic organic xenobiotics and, in particular, the halogenated organics continue to be a major area of attention and research emphasis in the Laboratory.

SUPPORT ACTIVITIES

Environmental chemistry interfaces environmental analysis, detection and measurement of environmental agents, with environmental toxicology, detection and measurement of various deleterious biological activities of environmental agents. Therefore, chemists are frequently consulted within NIEHS to advise biological scientists in appropriate areas of their research. The support activities of the Laboratory of Chemistry, as expected, fluctuate depending upon the resources required to meet a particular need. The requests generally fall into one of two categories: short term requests such as compound purity determinations 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 limitations 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.

Chemistry support generally involves the use of established known methodologies as opposed to the development of new ones. New methods development will be considered when it appears to be particularly relevant to ongoing programs and is appropriate to the expertise of the group. Special consideration is given to requests that cannot be done outside NIEHS or where clearly we can do it better. In addition, Chemistry provides support in securing procurement and research contracts of a chemical nature. Chemistry support requests are considered by one of three work groups. These work groups also form the basis for research activities within the Laboratory; viz. Analytical Biochemistry, Bioorganic Chemistry and Synthesis, and Specialty Instrumentation.

The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

The gas chromatography/mass spectrometry (GC/MS) facility continues to make the largest number (about 1800) of sample runs for analyses. About 24% of these was for routine service request, and the remaining in collaborative support of various biological studies and instrumental and method development research. A large percentage (over 50%) of the total runs are being performed using medium to high resolution mass spectrometry with data systems reflecting an increased use and dependence on the more sophisticated systems. A new mass spectrometer, the VG Micromass 7070, was added this reporting period which made such techniques as positive and negative chemical ionization and field desorption much more routine. Compounds analyzed covered a wide range of chemical classes, stabilities and volatilities.

High pressure liquid chromatography (HPLC) is being increasingly used by Laboratory personnel both within and outside this Laboratory. Because of this increased use and dependence in the various research programs, it is no longer practical to maintain an HPLC system for routine use by Institute staff.

The nuclear magnetic resonance (NMR) facility handled a number of different samples including a variety of chemical and biological metabolites and custom synthesized chemicals for biological/toxicological testing. In many of these compounds, both ^{1}H and ^{13}C nuclei using ^{1}H decoupling and fourier transform (FT) techniques were observed. Most of these samples were generated from projects within the Laboratory; the majority of those from outside the Laboratory are derived from projects within the Laboratory of Environmental Biophysics. The research NMR spectrometer is currently being operated continuously. However, instrument time needed to support other Institute collaborative programs continues to increase. A new FT-80 NMR spectrometer will soon be installed and should help alleviate this problem.

New and repeat synthesis of various halogenated hydrocarbons (especially the PCB's) for testing purposes continues to be a major involvement of the synthetic group. Other efforts involve further purification, characterization and identification of compounds available from other sources within and outside the Institute.

A number of other requests were made for analytical service which required specific methods. These ranged from specific analysis for various biological entities such as lipid classes and fatty acids, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to determination of organic vapors in air for the Institute safety program.

Chemical management services continue to facilitate the procurement and safe handling of specialty research chemicals during storage, analysis, testing and disposal. These activities are in general support of the National Toxicology Program and the mutagenicity testing program in particular.

RESEARCH ACTIVITIES

The nature of the research in the Laboratory of Environmental Chemistry is to some degree, predetermined by its support commitment and space limitations. For example, the Laboratory attempts to provide broad areas of science competence rather than indepth and narrow science expertise. However, this requires the availability of diverse instrumentation which consumes space and requires routine maintenance. There are five major areas of research involvement in the Laboratory which are using selected classes of chemical compounds as models and/or special chemical, biochemical and instrumental techniques to develop general methodology of value to environmental health scientists.

Immunochemistry and Immune Functions:

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. Radioimmunoassays for chlorinated dibenzo-p-dioxins, -biphenyls, and -dibenzofurans have been developed, as has a radioimmunoassay for mono-2-ethyl-hexyl phthalate in plasma. Development of a radioimmunoassay for 2,4,5-trichlorophenoxyacetic acid has encountered unexpected difficulties. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants. A contract effort for validation and refinement of these procedures has been initiated. The reproducibility of the radioimmunoassay for chlorinated dibenzo-p-dioxins in other laboratories has been demonstrated. Studies to evaluate the reliability of these assays in terms of false positives/false negatives are in progress.

Ingestion of many halogenated aromatic hydrocarbons and other environmental agents 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. Employing colony growth assays, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced bone marrow toxicity was found at dosage levels much lower than previously reported to occur. TCDD immunosuppression is due to a functional defect on the lymphocytes rather than elaboration of suppressor factors. TCDD exposure decreases resistance to tumor cell challenge and tumor development following injection of tumor cells. TCDD can directly inhibit lymphocyte functions following in vivo perfusion into the spleen through the splenic artery.

Other studies indicated that pharmacologically relevant doses of diethylstilbestrol (DES) (used therapeutically in humans) can suppress specific immunity while augmenting macrophage activity. 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.

Further selected halogenated aromatic hydrocarbons will be examined to determine their immunosuppressive potential (if such occurs) and their mechanism of immunosuppression. Using the in vitro model previously described, energy metabolism will be examined.

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 a host-resistance model (ability of the host to mount an immune response of the degree required to eliminate a gut-dwelling parasitic worm) to compare their sensitivity with assays of host resistance already employed at NIEHS and to evaluate their potential as useful tests of host resistance.

Mechanism Elucidation:

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.

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. 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 in three or more lateral positions. 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 high 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.

Structure-activity work (collaborative study with Oregon Regional Primate Center) with the hexachlorobiphenyls (HCBs) as model PCBs of high and constant chlorine content has been extended to include the monkey as a test species. A pilot experiment in male monkeys with three HCBs (2,4,5,2',4',5'- 2,4,6,2',4',6'- and 3,4,5,3',4',5'-) has been completed and the main experiment is to begin shortly.

The effects of single oral doses of 3,3',4,4',5,5'-, 2,2',4,4',5,5'-, and 2,2',3,3',5,5'-hexachlorobiphenyls on lipid metabolism in the rat have been studied relative to levels of activity of fatty acid CoA ligase, glycerol-

3-phosphate acyl transferase, diacylglycerol acyl transferase, diacyl glycerol choline phosphotransferase, lysophosphatidic acid acyl transferase and phosphatidic acid phosphohydrolase. In addition, the effects of the above PCBs on microsomal drug-metabolizing enzymes and on the electrophoretic properties of cytochrome P-448/450 have been examined. The general goal of these studies is to relate PCB structure to some of its mechanisms of action. These studies indicate that the coplanar and noncoplanar isomers have differential action.

The ability of such PCB isomers to induce cytochrome P-450, P-448, both or neither will be compared to their distortion polarizabilities, molar polarizations, and ionization potentials. These physical properties will be determined from measurements of dielectric constants, refractive indices, densities, molar volumes, charge transfer donar strengths, and London dispersion interactions. Knowledge of both the molecular geometry from X-ray diffraction studies, and the polarization properties from the current studies should elucidate the receptor site binding requirements involved in the four types of biological effects mentioned, and provide predictive ability.

These and related aromatic compounds are known to be metabolized to oxygenated derivatives via reactive intermediary arene oxide metabolites. Such processes can be both toxifying (activating) as well as detoxifying to the biological system. The abundant glutathione S-transferase enzyme system is being studied as a presumed important detoxifying pathway for such compounds. 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. Such mechanistic elaboration of the process will aid in understanding, preventing, and predicting the mechanism(s) of action of these compounds.

Other work includes evaluating 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. Of particular interest is understanding how enzymes in general are able to bind to and react with water-insoluble compounds. A new procedure for the purification of non-specific lipase has been developed, permitting its preparation on a larger scale than was previously possible. Its interaction with cofactors and substrates is being studied by circular dichroism and fluorescence spectroscopy. Circular dichroism studies have revealed that the processes of activation of pancreatic non-specific lipase are accompanied by increased α-helical content of the protein, whether activation involves ionic strength changes or exposure to bile salts. Inhibition by excess bile salt results in increased random coil content. β-Structure is apparently constant.

Further studies must await the purification of a larger amount of enzyme. One environmentally significant substrate for these enzyme systems is the phthalate ester plasticizers. Mass spectral CID processes have been used to confirm the identification of ten metabolites of diethylhexyl phthalate (DEHP), which have been isolated in small quantities as reference standards for metabolism studies. Plans are underway (at Letterman Army Institute of Research-LAIR) to use ^{13}C-labeled DEHP for studies of plasticizer pharmacokinetics in man. The role of NIEHS will be solely to analyze plasma and urine samples provided by LAIR. No work on this project was done this year due to the delay in LAIR obtaining FDA approval for use of human subjects.

Mechanistic work in the area of bioinorganic chemistry has been initiated. An initial literature survey has resulted in a report which describes some speciation and mechanistic aspects of trace metals in biological systems. Special research projects are focusing on the mechanistic aspects of trace metal interactions in defined biological systems. Preliminary work is investigating the effects of trace heavy metals (0.01-1 ppm) on the apparent activity of certain enzymes, particularly ATP-dependent enzymes and dehydrogenases, both of which comprise as much as 20% of the known enzymes in the living system. Preliminary results indicated that ATP-heavy metal complex is a competitive inhibitor of ATP-Mg^{++} in many ATP-dependent enzymes. Low concentrations of heavy metal ions were found to cause enzyme inhibitions by covalent interactions with the essential cysteinyl SH group in the active sites of enzymes. Unexpectedly, stoichiometric amount of reduced glutathione (GSH) (1:1 with respect to metal ions) reverses the enzyme inhibition caused by heavy metal ions. It is proposed that GSH can be a natural chelator for numerous metal ions in vivo.

Related work is examining two purified isoproteins of metallothioneins from liver of 100 rats that had been pretreated with Cd^{++}. Heavy metal ions, such as Cu^{++}, Zn^{++}, Hg^{++}, Ag^{+} and Pb^{++} were selected to replace Cd^{++} in purified metallothioneins to form different thionein-metal complexes. Proton and C^{13} NMR spectroscopy were employed to investigate the apparent structure differences of different thionein-metal complexes in an attempt to understand their physiological roles as metal binding proteins. Chemical model systems for investigating the binding of metals in biological systems are also being used. For example, the binding of lead to crown ethers, thio, and azasubstituted crown ethers and cysteine has been examined using ^{207}Pb NMR.

Mass Spectral (MS) Methods Development:

Mass spectrometry (in combination with gas chromatography) is still the most sensitive, specific and versatile analytical method available for dealing with complex samples of environmental/biological origins. Negative chemical ionization MS techniques are continuing to be developed for the more sensitive and specific analysis of environmental agents. This work was extended to include various nitriles including chlorinated, aliphatic and/or aromatic compounds. The compounds were shown to have characteristic fragmentations sensitive to the nature of the substitution pattern and, in representative cases, to be detectable at lower limits than with conventional positive ion chemical ionization mass spectrometry. Employing chemical

ionization methods, the first definitive experimental evidence in support of the monomeric phosphate anion was obtained.

Field-free region studies with selected environmental agents using high resolution mass spectrometry with metastable scanning has shown considerable promise in providing specific structural information useful in residue analysis as well as in establishing "chemical appearances" of environmental agents. Computer programs have been developed and refined which permit data system acquisition and processing of ion kinetic energy spectra. Mass-analyzed ion kinetic energy spectra of simple $C_5H_5^+$ and $C_5H_5^-$ ions were used to demonstrate that the frequently proposed cyclopentyl cation is not an important structure for $C_5H_5^+$ ions. Factors affecting dynamic range in "direct mixture" analysis using MIKES were explored. Usable dynamic range is limited by background secondary ion currents and source defocusing to ca. 10^4:1. Reasonably simple systems are being studied to provide the basic information necessary to understand the fragmentation reactions of highly energetic negative ions. The use of field free region reactions (usually after collisional activation) is being proposed for the analysis of crude mixtures.

Studies in field desorption mass spectrometry should illuminate the micro-environments important in environmental chemical carcinogenesis. Several derivatized deoxyribodinucleotides have been prepared and analyzed by field desorption mass spectrometry. Future work will attempt to find the best FD conditions to analyze the derivatives to provide spectra with maximum information content.

Molecular orbital calculations are continuing to be done to determine minimum energy configurations of neutral molecules and positive and negative ions in support of mass spectral analysis of compounds of environmental interest.

Other Analytical Methods Development:

It is often necessary 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. Classes of compounds studied have included the chlorinated dibenzo-p-dioxins and dibenzofurans, the polychlorinated and polybrominated biphenyls and their related and associated classes such as the chlorinated benzenes, phenols, diphenylethers and biphenyl dimers, some of which are formed under actual use conditions of commercial mixtures. Sample types have included liver, serum, milk, milk substitutes and adipose tissue from rodents, cows, monkeys, and humans. Studies of used fluids such as PCB based transformer and heat exchange (from Yusho oil) fluids have afforded methods for class separation of such compounds in complex mixtures. The analytic methods were developed using gas chromatography, thin-layer and column chromatography, fluorometry,

spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods were employed where necessary.

Nuclear magnetic resonance (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. Investigations are underway to apply NMR spectroscopy to problems of environmental significance and to examine the usefulness of NMR for such problems. ^{1}H and ^{13}C NMR spectra of all the possible chlorinated phenols have been obtained and interpreted as models for the polychlorinated dibenzofurans and dibenzodioxins In anticipation of future interest in the metabolism of heterocyclic polycyclic aromatic hydrocarbons (PAHs), ^{1}H and ^{13}C NMR spectra of several azaaromatics have been obtained. The results provided trends in the NMR parameters which should prove useful in the identification of unknown heterocyclic PAHs. Oxidative metabolites of several PAHs have been examined by ^{1}H and ^{13}C NMR for use as model compounds to develop substituent parameters for use in the identification of metabolites of larger PAHs. ^{1}H and ^{13}C NMR were also effectively used to characterize toxic fungal metabolites isolated from molds.

The initial development of a high pressure liquid chromatography (HPLC) separation for the glutathione conjugates from styrene oxide led to further studies on the effect of pH and ionic strength on the resolution of these amino acid derivatives. It was determined that resolution was better at pH 3 or pH 4, satisfactory at pH 7, and marginal at pH 5 or pH 6. The analysis times, however, are considerably shorter at pH 7 than at pH 3. An increase in buffer ionic strength resulted in increased retention times. These findings have found application in the preparative purification of conjugates prepared by the crown ether procedure.

Synthetic Methods Development:

Expertise in synthetic organic chemistry is highly desirable for supporting a variety of research programs at the Institute. Not only are a diverse nature of custom organic chemicals required but they must also be of high purity. Some of these chemicals are also highly toxic (or potentially) and require rather elaborate laboratory design facilities and equipment for maximum compound containment and control. Several potentially toxic brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs have been synthesized and characterized. The 2,3,6,7-tetra isomer was prepared from the known tetrakis (trimethylsilyl) naphthalene, the 1,2,3,5,6,7-hexa isomer from bromination of endo-2,3-trimethylenenorbornane and the 1,2,4,6,7-penta and 1,2,3,4,6,7-hexa from bromination of naphthalene. One or more of these compounds may be modified to provide heptenic compounds for radioimmunoassay purposes.

Other studies have involved the development of rather unique synthetic methodologies to permit preparation of specifically labeled compounds and unstable and/or reactive compounds which may represent intermediary metabolites. The new reactions for the synthesis of polynuclear aromatic hydrocarbons (PAH) uncovered last year have been exploited to prepare a number of benzanthracene derivatives. These have included oxygenated derivatives

of interest such as benzanthracene metabolites; alkylated derivatives of value to test current theories that correlate PAH structure with their carcinogenic activity; carbon-13 benzanthracene derivatives labeled specifically at one position of importance in the study of the metabolism of benzanthracene; derivatives with potential for elaboration to more complex structures heretofore not available or only obtainable with great effort.

Related work has developed a procedure for the synthesis of glutathione conjugates of arene oxides. The key step in this synthesis involves the use of a crown ether to dissolve glutathione in suitable organic solvents. The synthesis is also applicable to the preparation of conjugates from cysteinylglycine, cysteine and N-acetylcysteine. The scope of this procedure will be explored to include other alkylating agents of biological interest. Additionally, this synthetic route may allow for the first time, the preparation of conjugates with "abnormal" stereochemistry; i.e. cis as opposed to the accepted trans stereochemistry. Two objectives would be pursued with these cis conjugates; first, to conclusively establish the stereochemistry of the enzymatically produced thioethers; second, to study the effect, if any, of the abnormal stereochemistry in the biotransformation and excretion of glutathione conjugates.

OTHER ACTIVITIES

The Laboratory of Chemistry continues to provide unique analytical chemistry support to the study of environmental contamination problems of imminent public health concern. Through contract analytical chemistry work the Laboratory continues to support the epidemiological study to evaluate the health effects on infants of specific components of infant foodstuffs, particularly chlorinated pesticide residues and polychlorinated biphenyls (PCBs) in breast milk. This study requires well designed sample collection, analysis and data interpretation schemes. Contractors have completed development and validation studies for analyses in human milk, blood (serum) and placenta tissue and analysis of the first sets of mother's samples has been initiated. In developing these methods, it was shown that extensive method validation is required for each sample matrix of interest. Literature methods were of little value in facilitating the validation work. Reproducibility of methods is clearly a function of both method technology and operational techniques. Similar problems were encountered in a contract effort to develop a rapid screening analysis for aromatic hydrocarbon residues in diverse matrices. Similarly, methods have been developed and validated for polybrominated biphenyls (PBBs) in biological samples.

The Laboratory continues to receive and fulfill requests from organizations both nationally and internationally to provide analysis of important samples by unique methods developed here. Some of these samples were taken from humans (blood and adipose tissue).

Other contract work has completed the synthesis of high specific activity labeled (^{14}C) 2,3,7,8-tetrachlorodibenzofuran for biological study with respect to species differences in adsorption, metabolism, distribution, and excretion. The furans are associated highly toxic (relatedly) impurities of the commercial PCB preparations. The problems associated with this very

difficult synthesis were interpreted in terms of the intervention of "hot" free radical intermediates. The effects observed in the promotion of reactive free radical formation are perhaps not widely known.

Invitations to present lectures, teaching assignments at local universities and nominations to standing committees are accepted without rancor and are usually followed by active participation. The Laboratory is striving hard to establish a rapport with the various pertinent departments of the local universities in order to further enhance its research productivity and provide professional growth. In addition to supporting a continuing seminar program with invited speakers, Laboratory scientists have also organized specialized workshops/symposia to remain abreast of current methodology and other developments in their field.

The Laboratory remains cognizant of the Institute safety program. A special hazardous chemical containment facility for the Laboratory is now used routinely. The facility now permits a variety of chemical operations involving hazardous chemicals to be conducted with the minimum exposure possible to laboratory personnel. The Laboratory Office continues to cooperate with the Safety Office in providing chemistry input as needed. The Chief of the Laboratory continues to serve as Chairman of the Safety Committee.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30003-08 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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	0.7	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)

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.

PHS-6040
(Rev. 10-76)

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: Convenient methods have been developed for the extraction of lipophilic compounds from soil and adipose tissue. Extensive validation studies are in progress relative to recovery of various compounds of interest. Methods for the recovery of PCBs and DDE from human milk and serum have been studied, in order to accomplish reproducible extraction and cleanup without the use of halogenated or toxic solvents. The application of capillary column gas chromatography using uncommon liquid phases for the determination of trace amounts of halogenated aromatic pollutants is under investigation. Since much of the effort in this project area is in response to service 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.: Problems in analytical methodology. Sample handling, extraction and cleanup. Ann. N.Y. Acad. Sci. 320: 19, 1979.

Kohli, K.K., Albro, P.W., and McKinney, J.D.: Radioisotope dilution assay (RIDA) for the estimation of polychlorinated biphenyls. J. Anal. Toxicol. 3: 125, 1979.

Albro, P.W., Hass, J.R., and Crummett, W.B.: Summary of the Workshop on Recent Advances in Analytic Techniques for Halogenated Aromatic Compounds. Ann. N.Y. Acad. Sci. 320: 125, 1979.

Albro, P.W.: Validation of extraction and cleanup procedures for environmental analysis. In McKinney, J.D. (Ed.): The Chemistry of Environmental Agents as Potential Human Hazards. Ann Arbor Science, Ann Arbor, Michigan (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30012-07 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Pure Isomers of Hexachlorobiphenyls on Lipid Metabolism and Cell Structure

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

PI:	K. Kohli	Visiting Fellow	LEC NIEHS
OTHER:	P. Albro	Research Chemist	LEC NIEHS
	B. Gupta	Veterinary Medical Officer	EBB NIEHS
	H. Mukhtar	Visiting Associate	LP NIEHS
	R. Philpot	Research Chemist	LP NIEHS
	J. McKinney	Supervisory Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, CMB; and Marine Pharmacology Section, LP

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	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 effects of single oral doses of 3,3',4,4',5,5'-; 2,2',4,4',5,5'-; and 2,2',3,3',5,5'-hexachlorobiphenyls on lipid metabolism in the rat have been studied relative to levels of activity of fatty acid-CoA ligase, glycerol-3-phosphate acyl transferase, diacylglycerol acyl transferase, diacyl glycerol choline phosphotransferase, lysophosphatidic acid acyl transferase and phosphatidic acid phosphohydrolase. In addition, the effects of the above PCBs on microsomal drug-metabolizing enzymes and on the electrophoretic properties of cytochrome P-448/450 have been examined. The general goal of these studies is to relate PCB structure to some of its mechanisms of action. These studies indicate that the coplanar and noncoplanar isomers have differential action.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard lipid chemistry analytical methods, oral feeding of PCBs in corn oil by gavage, routine enzyme assays, histologic examination.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of single oral doses of 3,3',4,4',5,5'-, 2,2',4,4',5,5'-, and 2,2',3,3',5,5'-hexachlorobiphenyls on lipid metabolism and drug metabolizing enzymes have been studied 72 hr after dosing in male rat. The results suggest the hypothesis that the significantly coplanar 3,3',4,4',5,5'-HCB exerts its effects through its interaction with the Ah locus like 3-MC and TCDD whereas the two noncoplanar isomers do not. This study has been terminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies, and related studies of structure-activity relationships for the family of PCB compounds will contribute to our ability to predict the hazards of previously untested PCB-like compounds. This is desirable because of the ubiquitous nature of PCB pollution of the environment and the variety of PCB isomers and related compounds involved.

PUBLICATIONS

Kohli, K., Gupta, B.N., Albro, P.W., Mukhtar, H. and McKinney, J.D.: Biochemical effects of pure isomers of hexachlorobiphenyl. Fatty Livers and Cell Structure. Chem.-Biol. Interact. 25: 139, 1979.

Kohli, K., Mukhtar, H., Bend, J., Albro, P., and McKinney, J.: Biochemical effects of pure isomers of hexachlorobiphenyl - Hepatic microsomal epoxide hydrase and cytosolic glutathione S-transferase activities in the rat. Biochem. Pharmacol. 28: 1443, 1979.

Kohli, K., Philpot, R., Albro, P. and McKinney, J.: Induction of different species of cytochrome P-450 by coplanar and noncoplanar isomers of hexachlorobiphenyl. Life Sciences 26: 945, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30015-06 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 first definitive experimental evidence in support of the monomeric phosphate anion was observed. The negative ion mass spectra of various nitriles, including chlorinated, aliphatic and/or aromatic compounds were obtained and interpreted. Chemical ionization techniques have provided information complementary to that obtained using electron impact ionization methods.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromatography/mass spectrometry techniques, negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Various nitriles were studied by negative chemical ionization mass spectrometry. Exact mass measurements were performed by means of high resolution mass spectrometry. Unimolecular and collision-induced-decompositions were used to give ion structural information.

The compounds were shown to have characteristic fragmentations sensitive to the nature of the substitution pattern and, in representative cases, to be detectable at lower limits than with conventional positive ion chemical ionization mass spectrometry. Correlations of substitution pattern with specific reactions observed in negative ion chemical ionization mass spectrometry were made for these compounds which will act as guides to understanding the specificity of certain losses and ion-molecule reactions. The negative ion chemical ionization mass spectra of these compounds were shown to depend on the site of substitution and other structural features; several unprecedented types of ion-molecule reactions were observed.

This project will be extended by the study of the CI mass spectra of other environmental hazards.

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., Bursey, M.M., and Hass, J.R.: Methane enhanced negative ion mass spectra of organic nitriles. Org. Mass Spectrom. (in press).

Harvan, D.J., Hass, J.R., Busch, K.L., Bursey, M.M., Ramirez, F. and Meyerson, S.: Direct observation of the monomeric metaphosphate anion. J. Am. Chem. Soc. 101, 7409-7410, 1979

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30018-07 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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. A new procedure for the purification of nonspecific lipase has been developed, permitting its preparation on a larger scale than was previously possible. Its interaction with cofactors and substrates will be studied by circular dichroism and fluorescence spectroscopy.

PHS-6040
(Rev. 10-76)

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: Circular dichroism studies have revealed that the processes of activation of pancreatic non-specific lipase are accompanied by increased α-helical content of the protein, whether activation involves ionic strength changes or exposure to bile salts. Inhibition by excess bile salt results in increased random coil content. β-structure is apparently constant. Further studies must await the purification of a larger amount of enzyme.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30020-09 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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, LAIR

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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)

CID mass spectral processes have been used to confirm the identification of ten metabolites of diethylhexyl phthalate (DEHP), which have been isolated in small quantities as reference standards for metabolism studies. Plans are underway (at LAIR) to use ^{13}C-labeled DEHP for studies of plasticizer pharmacokinetics in man. The role of NIEHS will be solely to analyze plasma and urine samples provided by LAIR. No work on this project was done this year due to the delay in LAIR obtaining FDA approval for use of human subjects.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The previously isolated (rat) metabolites of DEHP have been unequivocally identified by mass spec collisionally induced dissociation (CID) methods. The proposed study of ^{13}C-phthalate pharmacokinetics in man is delayed while the necessary permission to use human subjects is obtained by LAIR.

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. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30034-05 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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: None

COOPERATING UNITS (if any)

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Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	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.

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, hplc).

MAJOR FINDINGS AND PROPOSED COURSE: The new reactions for the synthesis of polynuclear aromatic hydrocarbons (PAH) uncovered last year have been exploited to prepare a number of benzanthracene derivatives. These have included oxygenated derivatives of interest such as benzanthracene metabolites; alkylated derivatives of value to test current theories that correlate PAH structure with their carcinogenic activity; carbon-13 benzanthracene derivatives labeled specifically at one position of importance in the study of the metabolism of benzanthracene; derivatives with potential for elaboration to more complex structures heretofore not available or only obtainable with great effort.

It is proposed to continue the development of new synthetic approaches to polynuclear aromatic hydrocarbons and their metabolites such as arene oxides and hydroxylated derivatives.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polynuclear aromatic hydrocarbons are recognized to be of major environmental importance due to their widespread ecological distribution and, in some cases, their concentration in the food chain. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit critical biological and toxicological studies. Synthetic availability of appropriate model compounds allows further examination of the mechanisms of their biological activity.

PUBLICATIONS

Levy, L.A. and Pruitt, L.: An expeditious synthesis of benz(a)anthracene and some of its oxygenated derivatives. J. Chem. Soc. Chem. Comm. 227, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30035-05 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Chemical Techniques and Spectroscopic Methods

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
	P. A. Albro	Research Chemist	LEC NIEHS
	J. R. Hass	Research Chemist	LEC NIEHS
	R. H. Cox	Research Chemist	LEC NIEHS
OTHER:	K. C. Chae	Chemist	LEC NIEHS
	L. A. Levy	Research Chemist	LEC NIEHS
	C. E. Parker	Research Chemist	LEC NIEHS
	D. B. Walters	Technical Programs Coordinator	LEC NIEHS

COOPERATING UNITS (if any)
NCI Chemical Repository.
Others as needed.

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	1.6	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This general project has as its objective to employ chemical techniques, both analytical and synthetic, and spectroscopic methods, in particular gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy (NMR) and high pressure liquid chromatography (HPLC), to the preparation, determination and characterization of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general compounds covered by this study will include those for which adequate techniques and methods are already available or can be adapted. Chemical management services are also performed in support of various research programs.

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytical methods emphasized in this study are GC/MS, NMR or fourier transform NMR and HPLC. A variety of synthetic techniques are employed. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: The GC/MS facility continues to make the largest number (about 1800) of sample runs for analyses. About 24% of these was for routine service request, and the remaining in collaborative support of various biological studies and instrumental and method development research. A large percentage (over 50%) of the total runs are being performed using medium to high resolution conditions with data systems reflecting an increased use and dependence on the more sophisticated systems. A new mass spectrometer, the VG Micromass 7070, was added this reporting period which made such techniques as positive and negative chemical ionization and field desorption much more routine. Compounds analyzed covered a wide range of chemical classes, stabilities and volatilities.

High pressure liquid chromatography (HPLC) is being increasingly used by laboratory personnel both within and outside this laboratory. Because of this increased use and dependence in the various research programs, it is no longer practical to maintain an HPLC system for routine use by Institute staff.

The nuclear magnetic resonance (NMR) facility handled a number of different samples including a variety of chemical and biological metabolites and custom synthesized chemicals for biological/toxicological testing purposes. In many of these compounds, both ^{1}H and ^{13}C nuclei using ^{1}H decoupling and fourier transform (FT) techniques were observed. Most of these samples were generated from projects within the Laboratory; the majority of those from outside the Laboratory are derived from projects within the Laboratory of Environmental Biophysics. The research NMR spectrometer is currently being operated continuously. However, instrument time needed to support other Institute collaborative programs continues to increase. A new FT-80 NMR spectrometer will soon be installed and should help alleviate this problem.

New and repeat synthesis of various halogenated hydrocarbons (especially the PCB's) for testing purposes continues to be a major involvement of the synthetic group. Other efforts involve further purification, characterization and identification of compounds available from other sources within and outside the Institute.

A number of other requests were made for analytical service which required specific methods. These ranged from specific analysis for various biological entities such as lipid classes and fatty acids, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to determination of organic vapors in air for the Institute safety program.

Chemical management services continue to facilitate the procurement and safe handling of specialty research chemicals during storage, analysis, testing and disposal.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30041-05 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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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.

PHS-6040

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: That the radioimmunoassay for chlorinated dibenzo-p-dioxins can be successfully reproduced in other laboratories has been shown. A procedure for the radioimmunoassay of chlorinated dibenzofurans has been developed. The furan assay is more specific than the dioxin assay reported previously, but this specificity is a function of the antisera production of a minority of rabbits tested. The extreme storage stability of these antisera continues to be observed. Studies to evaluate the reliability of these assays in terms of false positives/false negatives are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Albro, P.W., Luster, M.I., Chae, K., Chaudhary, S.K., Clark, G., Lawson, L.D., Corbett, J.T., and McKinney, J.D.: A radioimmunoassay for chlorinated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 50: 137, 1979.

Luster, M.I., Albro, P.W., Clark, G., Chae, K., Chaudhary, S.K., Lawson, L.D., Corbett, J.T., and McKinney, J.D.: Production and characterization of antisera specific for chlorinated biphenyl species. Initiation of a radioimmunoassay for Aroclors®. Toxicol. Appl. Pharmacol. 50: 147, 1979.

Norstrom, A., Chaudhary, S.K., Albro, P.W., and McKinney, J.D.: Synthesis of chlorinated dibenzofurans and chlorinated amino-dibenzofurans from the corresponding diphenyl ethers and nitro-diphenyl ethers. Chemosphere 6: 331, 1979.

Luster, M.I., Albro, P.W., Chae, K. and McKinney, J.D.: Development of radioimmunoassays for chlorinated aromatic hydrocarbons. In McKinney, J.D. (Ed.): The Chemistry of Environmental Agents as Potential Human Hazards. Ann Arbor Science, Ann Arbor, Michigan (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30050-04 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	R. H. Cox	Research Chemist	LEC NIEHS
OTHER:	O. Hernandez	Visiting Associate	LEC NIEHS
	B. Smith	Staff Fellow	LP NIEHS
	J. Bend	Visiting Scientist	LP NIEHS
	B. Yagen	Visiting Scientist	LEC NIEHS
	J. D. McKinney	Supervisory Research Chemist	LEC NIEHS

COOPERATING UNITS (if any)

Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. NO1-CP-33387. Supplemented by NIEHS).

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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: Careful NMR and HPLC analysis of the conjugates of (+)- and (-)-styrene oxide with glutathione and N-acetylcysteine (mercapturic acid) have allowed the determination of the absolute stereochemistry of these isomers. Future studies include the synthesis of radiolabeled, ^{3}H or ^{14}C, optically pure isomers of styrene oxide and its glutathione conjugates and the use of these substrates for further metabolic studies. Of particular interest will be the relative rate of formation and excretion of the glutathione conjugates.

The metabolism of the glutathione conjugates of (±) styrene oxide by the rat and the 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 (±)-benzo[a]pyrene 4,5-oxide-4,5-^{13}C by a purified glutathione transferase from little river skate (Raja erinacea) liver demonstrated that equivalent amounts of the positional isomers (4,5-dihydro-4-hydroxy-5-glutathionylbenzo[a]pyrene and 4,5-dihydro-4-glutathionyl-5-hydroxybenzo[a]pyrene) were formed. Separation of these conjugates by HPLC and subsequent ^{13}C-NMR studies showed that only one diastereoisomer of each positional isomer was formed by the skate enzyme, each enantiomer of the arene oxide having produced only one of the two possible positional isomers. The data demonstrate that the purified skate liver glutathione transferase has high substrate regiospecificity and stereospecificity for (±)-benzo[a]pyrene 4,5-oxide. Future studies will include the use of the above HPLC procedure to analyze the regiospecificity and stereospecificity of the enzymatic conjugation of glutathione with benzo[a]pyrene 4,5-oxide catalyzed by purified glutathione transferases from various sources. The stereochemistry of the conjugation of glutathione with other arene oxides will be investigated.

Synthetic procedures which allow the preparation of mercapturic (N-acetylcysteine) derivatives and glutathione conjugates were developed. The generality of these methods was proven by synthesizing thioether derivatives from a number of alkene and arene oxides. Notably the latter included benzo[a]pyrene 4,5-oxide, a compound which was known to be metabolized by conjugation with glutathione. The same methodology has been used to prepare the corresponding S-alkylated cysteine and cysteinylglycine derivatives, and completing in this way the metabolic scheme by which glutathione conjugates are transformed to mercapturic acids.

The conditions originally developed for the separation of styrene oxide glutathione conjugates were refined to allow preparative purifications.

The use of volatile buffer salts made recovery of samples collected from the HPLC analysis more efficient and quantitative. Notable examples included the separation of stereoisomers such as the cysteine derivatives from β-methylstyrene oxide and glutathione conjugates of benzo[a]pyrene 4,5-oxide. In the former case, an interesting finding was the great disparity in the extinction coefficient values (254 nm) for the diastereomers (ε_1=300 vs ε_2=120). This finding indicates that caution should be exercised when attempting to quantify mixtures of diastereomeric thioether metabolites based exclusively on UV adsorbtion generated HPLC profiles.

^{1}H and ^{13}C NMR spectra have been recorded and partially analyzed for a number of polycyclic aromatic hydrocarbons and their derivatives. ^{13}C NMR spectra have been obtained and fully analyzed for the potential oxidative metabolites of acenaphthylene 1,2-oxide, phenanthrene 9,10-oxide and pyrene 4,5-oxide. These data will be useful in the identification of metabolites of other arene oxides.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Certain arene oxides are known to bind covalently to nucleic acids, and they have been implicated as carcinogens and mutagens which can be derived from the parent compounds. The abundant detoxifying glutathione S-transferase enzyme system is also likely to covalently bind these oxides. Such mechanistic elaboration of the process will aid in understanding, preventing, and predicting the mechanism(s) of action of these compounds.

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. (in press).

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. Commn. (in press).

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 (in press).

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 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30051-04 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	P. Singh	Research Chemist	LEC NIEHS
	L.A. Levy	Research Chemist	LEC NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	J.A. Goldstein	Pharmacologist	EBB NIEHS

COOPERATING UNITS (if any)

Comparative Biology Section
Oregon Regional Primate Center (Dr. McNulty)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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 high 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.

POSITION 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.

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.

Compounds tested this reporting period include 2,3,6,7-tetra-, 1,2,4,6,7-penta-, 1,2,3,5,6,7- and 1,2,3,4,6,7-hexabromonaphthalene. All of these compounds except the 1,2,3,5,6,7-hexabromonaphthalene were highly toxic to the guinea pig (LD_{50}, 100-200 times less toxic than 2,3,7,8-tetrachloro-dibenzo-p-dioxin). The lack of toxicity for the closely related 1,2,3,5,6,7-isomer was remarkable and again is thought to be related to its poor binding affinity for a specific biological receptor. These compounds are representative of the brominated naphthalene contaminants of the fire retardant chemicals polybrominated biphenyls (PBBs). Further work is examining the significance of the brominated naphthalenes in the toxicity of the PBBs.

Structure-activity work with the hexachlorobiphenyls (HCBs) as model PCBs of high and constant chlorine content has been extended to include the monkey as a test species. A pilot experiment in male monkeys with three HCBs (2,4,5,2',4',5'-, 2,4,6,2',4',6'- and 3,4,5,3',4',5'-) has been completed and the main experiment is to begin shortly.

The ability of various PCB isomers to induce cytochrome P-450, P-448, both or neither will be compared to their distortion polarizabilities, molar polarizations, and ionization potentials. These physical properties will be determined from measurements of dielectric constants, refractive indices, densities, molar volumes, charge transfer donar strengths, and London dispersion interactions. Knowledge of both the molecular geometry from X-ray diffraction studies, and the polarization properties from the current studies should elucidate the receptor site binding requirements involved in the four types of biological effects mentioned, and provide predictive ability.

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 McConnell, E.E.: Structural specificity and the dioxin receptor. Proceedings of the Workshop on TCDD and Related Compounds, October, 1980, Rome, Italy (Organized by the International Association of Environmental Analytical Chemistry).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30060-03 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 chaetoglobosin K and diplodiol from Diplodia macrospora, 20,26-dihydroxyaflavinine from A. flavus, pergillin from A. ustus and a sterol from Fusarium sporotrichioides 921.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: New compounds whose structures were determined include chaetoglobosin K and diplodiol from Diplodia macrospora, 20,26-dihydroxyaflavinine from A. flavus, pergillin from A. ustus and a sterol from Fusarium sporotrichioides 921. These compounds were either toxic to day-old cockerels and/or exhibited significant plant growth inhibitor properties. In addition, three new trichothecanes related to T-2 toxin have been isolated and their structures determined by NMR. A mold found growing in a can of commercial beer was cultured and three toxic indole metabolites were identified. One of the metabolites isolated earlier, cytochalisin H, is now in the second stage of testing by NCI as a tumor growth inhibitor.

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

Newton, M.G., Pautaleo, N.S., Churchill, F., and Cox, R.H.: The stereochemistry of Aflatoxicol B_1. J. Agric. Food Chem. 27: 1339, 1979.

Cox, R.H., Hernandez, O., Dorner, J.W., Cole, R.J., and Fennell, D.I.: A new isochroman mycotoxin isolated from Penicillium steckii. J. Agric. Food Chem. 27: 999, 1979.

Cutler, H.G., Crumley, F.G., Cox, R.H., Hernandez, O., Cole, R.J., and Dorner, J.W.: Orlandin: A non-toxic fungal metabolite with plant growth-inhibiting properties. J. Agric. Food Chem. 27: 592, 1979.

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. Phytochem. (in press).

Springer, J.P., Dorner, J.W., Cole, R.J., and Cox, R.H.: Terretoxin, a toxigenic compound from Aspergillus Terreus. J. Org. Chem. 44: 4852, 1979.

Cutler, H.G., Crumley, F.C., Cox, R.H., Cole, R.J., Dorner, J.W., and Latterell, F.M.: Diplodiol: A new toxin from Diplodia macrospora. J. Agric. Food Chem. 28: 135, 1980.

Cutler, H.G., Crumley, F.G., Cox, R.H., Cole, R.J., Dorner, J.W., Springer, J.P., Latterell, F.M., Thean, J.E. and Rossi, A.E.: Chaetoglobosin K: A new plant growth inhibitor and toxic from Diplodia macrospora. J. Agric. Food Chem. 28: 139, 1980.

Springer, J.P., Cox, R.H., Cutler, H.G. and Crumley, F.G.: The structure of chaetoglobosin K. Tetrahedron Lett. (in press).

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. (in press).

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. (in press).

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. (in press).

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. J. Agric. Food Chem. (in press).

Cutler, H.G., Cole, R.J., Cox, R.H. and Wells, J.M.: Fungal metabolites: Interesting new plant growth inhibitors. Presented at the 6th Annual Meeting of the Plant Growth Regulator Working Group, Las Vegas, 1979.

Cole, R.J. and Cox, R.H.: Handbook of Toxic Tungal Metabolites. Academic Press, New York (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30062-03 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 ☒ (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 in $B_6C_3F_1$ hybrid mice. Exposure was accomplished by maternal dosing on day 14 of gestation and again on days 1, 7, and 14 following birth employing dosages of 0, 1.0, 5.0 or 15.0 µg/kg body weight. The 15.0 µg/kg dosage was lethal to 70% of the offspring with the remainder of that dosage group revealing overt toxicity. Bone marrow toxicity occurred in both the 15.0 and 5.0 µg/kg dosage groups as evidenced by bone marrow hypocellularity and depressed colony formation of macrophage-granulocyte progenitor cells and pleuripotent stem cells. Evidence was presented that depression of lymphoproliferative responses following mitogen stimulation in TCDD-immunosuppressed mice was due to a functional defect of lymphocyte activation rather than suppressor cell activity. Administration of either Listeria monocytogenes or syngeneic PYB6 tumor cells in mice exposed to relatively low levels of TCDD during pre- and postnatal development increased their susceptability to either bacterial or tumor challenge.

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 *Listeria monocytogenes* and tumor development following injection of PYB6 tumor cells.

MAJOR FINDINGS AND PROPOSED COURSE: Employing colony growth assays, TCDD induced bone marrow toxicity was found at dosage levels much lower than previously reported to occur. TCDD immunosuppression is due to a functional defect of the lymphocytes rather than elaboration of suppressor factors. TCDD exposure decreases resistance to tumor cell challenge and tumor development following injection of tumor cells. TCDD can directly inhibit lymphocyte functions following *in vivo* perfusion into the spleen through the splenic artery.

Further selected halogenated aromatic hydrocarbons will be examined to determine their immunosuppressive potential (if such occurs) and their mechanism of immunosuppression. Using the *in vitro* model previously described (Luster, *et al.*, J. Environ. Path. & Tox. 2:965, 1979), energy metabolism will be examined.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Ingestion of many halogenated 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. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30063-03 LEC

PERIOD COVERED

October 1, 1979 to September 30, 1980

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:
0.1	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)

Molecular orbital calculations have been done to determine minimum energy configurations of neutral molecules and positive and negative ions with respect to the mass spectral analysis of compounds of environmental interest.

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: The INDO/CNDO/2 and MINDO/3 methods have been tried on the molecules of interest, after the programs were modified to handle the large number of atoms and the types of atoms needed for the study. The structures of negative as well as positive ions were studied using both molecular orbital methods. For certain halogenated compounds, non-convergence was found which seems to correspond to ionization to a dissociative state. For systems with a single aromatic ring, minimum energy geometries and relative energies predicted by these two programs seem to agree. Different results were obtained for two-ring systems, such as biphenyls, where preliminary results seemed to indicate that the INDO/CNDO/2 method may give invalid results due to the overestimate of non-bonding interactions. This project will be continued by investigating the use of these programs for other molecules of environmental interest.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30064-03 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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)

Several derivatized deoxyribodenucleotides have been prepared and analyzed by field desorption mass spectrometry. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis.

PROJECT DESCRIPTION

METHODS EMPLOYED: Field desorption mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Three of sixteen protected deoxyribodinucleotides d-(nucleoside)-1-phosphorus-(nucleoside)-2 synthesized in the course of building gene fragments, have been submitted to field desorption ionization on the ZAB/2F mass spectrometer, using activated emitters. We were successful only in the case where $(base)_1$=$(base)_2$=thymine in differentiating between the few detected ions, the most abundant ones are given by the thymine molecules (peak at m/z=126). This study will be continued by attempting to find the best FD conditions to analyze the rest of the derivatives and also by the use of other ionization techniques.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Finding mass spectrometric methods for the analysis of small polynucleotide-carcinogen 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 microenvironments important in environmental chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30065-03 LEC

PERIOD COVERED

October 1, 1979 to September 30, 1980

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

COOPERATING UNITS (if any)

M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC.

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Laboratory of Environmental Chemistry

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Mass-analyzed ion kinetic energy spectra (MIKES) of $C_5H_5^+$ and $C_5H_5^-$ ions were used to demonstrate that the frequently proposed cyclopentyl cation is not an important structure for $C_5H_5^+$ ions. Factors affecting dynamic range in "direct mixture" analysis using MIKES were explored. Usable dynamic range is limited by background secondary ion currents and source defocusing to ca. 10^4:1. Computer programs for MIKES analysis have been refined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: The study of collisional activation of negative ions is relatively undeveloped. Therefore, we are working with reasonably simple systems to provide the basic information necessary to understand the fragmentation reaction of highly energetic negative ions. Thus, positive and negative ions from $C_5H_5^-$ ions from various precursors were studied. Comparisons with the positive ionic fragments resulting from the collisional excitation of $C_5H_5^+$ ions from the same precursors lead to the conclusion that the cyclopentyl cation is not the structure of the $(M-H)^+$ in the mass spectrum of cyclopentane.

The use of field free region reactions (usually after collisional activation) is being proposed for the analysis of crude mixtures. A study of the scope and limitations of this technique for the determination of dioxins in tissue samples was undertaken. When >400 mg of material was in the ion source, sensitivity for the analyte (at the 100 pg level) began to decrease. With 1 µg, the response was ca. 67% the "true" value. With no analyte, signals corresponding to ca. 50 pg were observed. It was found that no reduction in sample clean-up was possible through use of this technique if accurate quantitation results were to be obtained.

This project will be continued by completing the above experiments. In addition, these techniques will be extended to other environmental chemicals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry (in combination with gas 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 structural analysis.

PUBLICATIONS

Hass, J.R. and Harvan, D.J.: Some experiences using MIKES in environmental analysis. Environ. Hlth. Perspect. (in press).

Bursey, M.M., Hass, J.R. and Harvan, D.J.: The nonexistance of gaseous cyclopentadienyl cations: An overdue correction to the literature using collision induced decompositions and charge stripping of negative ions. Tetrahedron Letts., 4725-4728, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30066-03 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	P. Singh	Research Chemist	LEC NIEHS
	L. Levy	Research Chemist	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	0.3	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)

Several brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs have been synthesized and characterized. These include the 2,3,6,7-tetra-, 1,2,4,6,7-penta- and 1,2,3,5,6,7- and 1,2,3,4,6,7-hexa isomers.

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: Several brominated naphthalenes representative of the naphthalene contaminants of the fire retardant chemicals PBBs have been synthesized and characterized. The 2,3,6,7-tetra isomer was prepared from the known tetrakis (trimethylsilyl) naphthalene, the 1,2,3,5,6,7-hexa isomer from bromination of endo-2,3-trimethylenenorbornane and the 1,2,4,6,7-penta and 1,2,3,4,6,7-hexa from bromination of naphthalene. One or more of these compounds may be modified to provide haptenic compounds 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.

PUBLICATIONS

McKinney, J.D., Singh, P., Levy, L., Walker, M., Cox, R., Bobenrieth, M.J. and Bordner, J.B.: Synthesis of some highly brominated naphthalenes, J. Agric. Food. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30067-03 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Characterization of the Binding of Heavy Metals in Biological Systems

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)
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	0.2	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, ^{13}C and metal nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the specific binding character of certain heavy metals using purified biopolymers, their monomer and other isolated and definable biological systems as models. An attempt is being made to correlate binding propensity with toxic effects of the heavy metals. Early work indicates that ^{207}Pb NMR offers a useful probe into the binding of lead.

PROJECT DESCRIPTION

METHODS EMPLOYED: Multi nuclei nuclear magnetic resonance (NMR) spectroscopy using biological models for studying the interactions between heavy metals. Isolation and characterization of specific binding site(s) in body tissue and fluid.

MAJOR FINDINGS AND PROPOSED COURSE: There is an increasing body of evidence that the transport and accumulation of heavy metals in body tissue and fluids is related to the binding of the metals in biological systems. Our previous studies 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 were elucidated. These studies have been continued with the examination of a number of organic ligand complexes with lead. In several cases, the exchange rate of lead 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 the intermediate 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 cysteine has been examined. Future studies will continue to examine the ^{207}Pb chemical shift changes upon binding of lead to a variety of ligand types and biological systems in an attempt to correlate binding propensity with toxicity. These studies will also be extended to include metals other than lead.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing evidence that certain heavy 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30074-02 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development of Program in Bioinorganic Chemistry

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:	R. H. Cox	Research Chemist	LEC NIEHS
	G. Lee	Senior Staff 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:
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)

A program in bioinorganic chemistry has been initiated. The initial literature survey has resulted in a report which describes some speciation and mechanistic aspects of trace metals in biological systems. Special research projects are focusing on the mechanistic aspects of trace metal interactions in defined biological systems.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard methods and procedures used to search the scientific literature and prepare scientific reports.

MAJOR FINDINGS AND PROPOSED COURSE: A program in bioinorganic chemistry is being initiated. Three stages of development have been considered, viz. (1) assessment of the state-of-the-art in inorganic residue analysis; (2) development of separation methodologies compatible with the selected analytical measurement techniques; (3) the application of validated methods to actual biological problems.

A report has been completed and will soon be published describing some speciation and mechanistic aspects of trace metals in biological systems.

This initial phase involved a great deal of literature searching, screening, etc. to assess the state-of-the-art in methods (both for speciation and elemental analysis), interaction with various Institute programs and scientists to find out what types of problems they are having in working with inorganics, e.g. heavy metals, assessment of the inorganics of greatest environmental concern, and interaction with the laboratories both private and academic to learn about their efforts in this area. For the immediate future, research in this area will focus on the mechanistic aspects of trace metal interactions in defined biological systems (see new projects Z01 ES 30075 and 30076). This project dealing with the initial literature work has been completed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relative to the organic residues in our environment, the inorganic residues have received little attention with regard to potential as human hazards. The environmental load of inorganics is expected to increase as a result of their being by-products of energy technologies. Development of a strong program in bioinorganic chemistry is essential to meeting this need.

PUBLICATIONS

Boline, D.: Some speciation and mechanistic aspects of trace metals in biological systems. In McKinney, J.D. (Ed.): Environmental Health Chemistry. Chemistry of Environmental Agents As Potential Human Hazards. Ann Arbor Science Publishing Co., Ann Arbor, Michigan, Chapter 24 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 10001 Z01 ES ~~30075~~-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Chemical Characterization of Metallothioneins from Rat Liver

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Two isoproteins of metallothioneins were purified to homogeneity from liver of 100 rats that had been pretreated with Cd^{++}. Heavy metal ions, such as Cu^{++}, Zn^{++}, Hg^{++}, Ag^{+} and Pb^{++} were selected to replace Cd^{++} in purified metallothioneins to form different thionein-metal complexes. Proton and C^{13} NMR spectroscopy were employed to investigate the apparent structure differences of different thionein-metal complexes in an attempt to understand their physiological roles as metal binding proteins.

PROJECT DESCRIPTION

METHODS EMPLOYED: G-75-Sephadex gel filtration chromatography and DEAE-Sephadex ion exchange chromatography were employed to purify metallothioneins to homogeneity from rat liver. Structure of different thionein-metal complexes will be investigated by UV and NMR spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: About 50 mg each of metallothionein I and II were purified from liver of 100 treated rats by two steps each of DEAE-Sephadex and G-75-Sephadex chromatography. They were shown to be homogeneous by acrylamide gel electrophoresis and have a molecular weight of about 6,000. Hg^{++}-thionein and other thionein-metal complexes were reconstituted at low pH. Proton and C^{13} NMR study of these metallothioneins is in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Metallothioneins have been implicated to function for the temporary storage and the detoxification of heavy metal ions. Through our study, we attempt to obtain information regarding the specificity and affinity of these metal binding proteins to various heavy metal ions commonly present in our environment. Structural analysis of metallothioneins should lead to a better understanding regarding its metabolic roles *in vivo*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES ~~30076~~ 1002-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biochemical Toxicity of Heavy Metals

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

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Chemistry

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The purpose of this research project is to investigate the effects of trace heavy metals (0.01-1 ppm) on the apparent activity of certain enzymes, particularly ATP-dependent enzymes and dehydrogenases, both of which comprise as much as 20% of the known enzymes in the living system. Preliminary results indicated that ATP-heavy metal complex is a competitive inhibitor of ATP-Mg^{++} in many ATP-dependent enzymes. Low concentrations of heavy metal ions were found to cause enzyme inhibitions by covalent interactions with the essential cysteinyl SH group in the active sites of enzymes. Unexpectedly, stoichiometric amount of reduced glutathione (GSH) (1:1 with respect to metal ions) reverses the enzyme inhibition caused by heavy metal ions. It is proposed that GSH can be a natural chelator for numerous metal ions in vivo.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: The following enzymes from mouse muscle were assayed spectrophotometrically according to the known procedures: adenylate kinase, pyruvate kinase, hexokinase, 6-phosphofructokinase, 3-phosphoglycerate kinase, lactate dehydrogenase, -glycerolphosphate dehydrogenase, aldolase, glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase. For ATP-dependent enzymes, the assays were performed in the presence of 1 mM Mg^{++}, 1 mM ATP and varying amount of metal ions. The following metal ions were selected for inhibition study (10^{-3} to 10^{-6}M): Hg^{++}, Cd^{++}, Zn^{++}, Cu^{++}, Al^{+++}, Cr^{+++}, Pb^{++} and Ag^{+}. Proton and C^{13} NMR spectroscopy will be employed as tool to study specific metal ion interactions with ATP or with reduced glutathione.

MAJOR FINDINGS AND PROPOSED COURSE: Most of the enzymes examined were greater than 90% inhibited in the presence of 1:1 ratio of metal ion to Mg^{++}. In the case of 3-phosphoglycerate kinase, glyceraldehyde dehydrogenase and hexokinase were 50% inhibited at Cu^{++}/Mg^{++} of 0.01. Similarly, greater than 50% inhibition of hexokinase activity was observed at Cd^{++}/Mg^{++} or Pb^{++}/Mg^{++} of 1/100. In almost all the cases examined, 1 mM reduced glutathione (GSH) reversed the enzyme inhibition and restored the enzyme activity. Other thiol reagents, such as mercaptoethanol and -thioglycerol are ineffective. This phenomenon was interpreted as the results of the chelation of heavy metal ions specifically by reduced glutathione. Specific chelation of heavy metal ions by reduced glutathione could be physiologically important for the rapid removal of heavy metal ions from the enzyme surface and for the prevention of enzyme inactivations. Detailed molecular mechanism of metal toxicity on these enzymes will be elucidated in this study.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Trace heavy metal ions are widely spread in our environment. Investigation of the effects of heavy metal ions on the activity of a variety of enzymes is important for the understanding of molecular mechanism of metal toxicity in humans. The results of this study seems to indicate that trace amount of heavy metal ions (0.01-1 ppm) have profound effect on numerous enzymes through either chelation with ATP or with essential SH group of enzymes. The dramatic effect of reduced glutathione seemed to point out the physiological importance of this natural chelating agent. Some of the pathological conditions in humans could be explained on the basis of enzyme inhibitions by trace metal ions *in vivo*. For example, Al^{3+} has been shown to inhibit brain hexokinase and has been implicated as the cause of encephalopathy (brain disease).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 10003 Z01 ES 30077-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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:
0.3	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 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.

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 initial development of a HPLC separation for the glutathione conjugates from styrene oxide led to further studies on the effect of pH and ionic strength on the resolution of these amino acid derivatives. It was determined that resolution was better at pH 3 or pH 4, satisfactory at pH 7, and marginal at pH 5 or pH 6. The analysis times, however, are considerably shorter at pH 7 than at pH 3. An increase in buffer ionic strength resulted in increased retention times. These findings have found application in the preparative purification of conjugates prepared by the crown ether procedure (see below).

A procedure was developed for the synthesis of glutathione conjugates of arene oxides. The key step in this synthesis involves the use of a crown ether to dissolve glutathione in suitable organic solvents. The synthesis is also applicable to the preparation of conjugates from cysteinylglycine, cysteine and N-acetylcysteine. The scope of this procedure will be explored to include other alkylating agents of biological interest. Additionally, this synthetic route may allow for the first time, the preparation of conjugates with "abnormal" stereochemistry; i.e. cis as opposed to the accepted trans stereochemistry. Two objectives would be pursued with these cis conjugates; first, to conclusively establish the stereochemistry of the enzymatically produced thioethers; second, to study the effect, if any, of the abnormal stereochemistry in the biotransformation and excretion of glutathione conjugates.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES ~~30078~~-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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)

Investigations are underway to apply nuclear magnetic resonance (NMR) spectroscopy to problems of environmental significance and to examine the usefulness of NMR for such problems. ^{1}H and ^{13}C NMR spectra of all the possible chlorinated phenols have been obtained and interpreted as models for the polychlorinated dibenzofurans and dibenzodioxins. In anticipation of future interest in the metabolism of heterocyclic polycyclic aromatic hydrocarbons (PAHs), ^{1}H and ^{13}C NMR spectra of several azaaromatics have been obtained. The results provided trends in the NMR parameters which should prove useful in the identification of unknown heterocyclic PAHs. Oxidative metabolites of several PAHs have been examined by ^{1}H and ^{13}C NMR for use as model compounds to develop substituent parameters for use in the identification of metabolites of larger PAHs.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{1}H and ^{13}C high-resolution nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: 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 have allowed the development of a set of additivity parameters for the effect of chlorine substitution on NMR spectra. It appears that additivity parameters accurately reproduce the proton-proton coupling constants obtained from ^{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 unknown polychlorinated aromatics. The additivity parameters for predicting ^{13}C chemical shifts appear to reproduce the chemical shifts with a reasonable degree of accuracy.

^{1}H and ^{13}C NMR spectra of 1-, 4- and 9-azaphenanthrene, 4-azapyrene, and 2-azafluoranthrene 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.

The epoxide, cis- and trans-diols, quinone and mercapturic acid conjugates of acenaphthylene, phenanthrene, and pyrene have been examined by ^{13}C NMR spectroscopy. The data obtained have been used to develop a set of substituent parameters for use in the identification of the oxidative metabolites of larger PAHs. By using these parameters, the chemical shifts of the metabolites of benzo[a]pyrene-4,5-oxide are predicted to a reasonable accuracy such that chemical shift assignments of these metabolites can be made. This set of substituent parameters should prove very useful in the identification of oxidative metabolites of larger PAHs.

Future studies will continue to develop NMR methods that may prove useful in studying problems of environmental significance.

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. As new NMR techniques are developed, we will evaluate these techniques for application to problems of environmental importance. The background data obtained during this reporting period should prove useful in our continuing studies of the metabolism of polycyclic aromatic hydrocarbons.

PUBLICATIONS

Cox, R.H. and Hamada, M.: A ^{13}C NMR investigation of restricted rotation and dimerization in p-substituted nitrosobenzenes. Org. Magn. Reson. 12: 322, 1979.

Cox, R.H. and Sankar, S.: ^{1}H and ^{13}C NMR studies of 7-azaindole and related compounds. Org. Magn. Reson. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES ~~30079~~ 1005-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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: 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:
0.5	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)

These studies were undertaken to ascertain the effects on the immune elimination of helminthic parasites from mice exposed to various chemicals of environmental concern including diethylstilbestrol, Fyrol, and orthophenyl phenol. Evaluation of adult worm longenity in exposed animals indicated that selected chemicals adversely affect the ability of treated hosts to respond normally to a helminthic infection.

PROJECT DESCRIPTION

METHODS EMPLOYED: Adult worm expulsion was evaluated by counting the number of adults remaining in the gut at selected times. Larvae counts were done by a whole body digestion method. Standard histopathologic techniques were followed for evaluation of tissue responses.

MAJOR FINDINGS AND PROPOSED COURSE: These studies indicate that adult exposure to diethylstilbestrol and Fyrol impairs the ability of the host to mount an immune response of the degree required to eliminate a gut-dwelling parasitic worm (Trichinella spiralis). It is generally accepted that the host factors responsible for immune expulsion resides in the cellular (T-lymphocyte mediated) arm of the immune response. On the other hand, none of the compounds evaluated to date affected the numbers of encysted muscle larvae; however, reduced T. spiralis fecundity is mediated largely by humoral (B-lymphocyte) immunity and other non-lymphocytic leukocytes.

A generalized, pan-mucosal inflammatory reaction in the small intestine accompanies T. spiralis infection. Examination of routine histologic preparations of small bowel revealed a decrease in the expected response in animals exposed to diethylstilbestrol.

Further studies are in progress utilizing specific Trichinella antigens in tests of T-lymphocyte-mediated host responses in chemically treated mice. In addition, other chemicals will be evaluated in this host-resistance model. Also in progress are studies of host resistance to murine malaria, using a strain of Plasmodium berghei of predictable and uniform lethality in 21-25 days in the $B_6C_3F_1$ mouse.

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 resistance already employed at NIEHS and to evaluate their 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, 1980 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30006-01 LEC

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

COOPERATING UNITS (if any)

Dr. J. Thigpen, Environmental Biology Branch
Dr. J. Haseman, Biometry Branch

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)

Female $B_6C_3F_1$ mice (6-8 weeks of age) were administered subcutaneous injections of diethylstilbestrol (DES) in corn oil at dosages of 0, 0.2, 2.0 or 8.0 mg/kg body weight for 5 days. Although leukocytosis occurred, there was severe depression of immune functions including antibody responses, delayed hypersensitivity responses, lymphoproliferative responses and numbers of splenic T-cells. Bone marrow examinations revealed hypocellularity and decreased proliferation (colony formation). In contrast, macrophage functions were augmented including production and release of supernatant factors capable of suppressing normal immune function. Host susceptibility studies indicated decreased resistance to endotoxin, bacterial infection, tumor cell challenge and parasitemia. In vitro treatment of spleen cells from DES animals with indomethacin inhibited the production of suppressor activity indicating that this factor is prostaglandin.

PROJECT DESCRIPTION

METHODS EMPLOYED: Immunity was examined by a variety of *in vitro* and *in vivo* functional assays including lymphoproliferative responses, antibody responses, delayed hypersensitivity, phagocytic index, macrophage cytostasis, etc. Host resistance assays included susceptibility to tumor cells, Listeria infection, Trichinella infection and endotoxin sensitivity. Values obtained in DES treated mice were compared to controls for statistical evaluation.

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. Furthermore, immune suppression correlates with decreased host resistance. Further studies are being conducted to determine the relationship of DES-induced carcinogenicity and immune alterations. Macrophage enzyme activity is being examined as well as *in vivo* treatment with indomethacin and aspirin.

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

Luster, M.I., Boorman, G.A., Dean, J.H., Lawson, L.D., Wilson, R. and Haseman, J.K.: Immune alterations in mice following acute adult exposure to diethylstilbestrol. In Dean, J.H. and Padarathsingh, M.L. (Ed.): Biological Relevance of Immunosuppression. Van Norstrand Reinhold, New York, 1980 (in press).

RALTECH SCIENTIFIC SERVICES - Madison, Wisconsin 60616
(NIH-NO1-ES-7-2141)

TITLE: Analysis of PCB's and DDE in Human Body Fluids and Tissue

CONTRACTOR'S PROJECT DIRECTOR: D.L. Hughes

PROJECT OFFICER (NIEHS): J.D. McKinney, Ph.D., Chief, LEC

DATE CONTRACT INITIATED: September 30, 1977

CURRENT LEVEL (3 years): $267,656

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-chlorobiphenyl)-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 and usual sample preparation, clean-up, extraction and lipid determination 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. Contract NIH-NO1-ES-8-2105 serves as a check and validation of the significance of the results. 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.

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 (3 years): $539,389

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 compatable with NAA measurements. Additional methodology has been developed for milk substitutes (formula) and placenta tissue extracts. Analyses have been performed on about 300 samples to date. It is anticipated that approximately 300 samples will be analyzed every 2 months.

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 validated. 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.

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.

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.

FLORIDA STATE UNIVERSITY - Tallahassee, Florida 32306
NO1-ES-9-0003

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: Due to changes in NIEHS priorities, the expected "targeted" research samples have not appeared. Therefore, we have utilized the contractors capabilities to test various extraction/cleanup methods currently in use. Results from the analysis of infant milk formula, human milk, rat livers and oysters spiked with at least one of the following: Halowax 1013; 3,4,3',4'-tetrachlorobiphenylene; used transformer fluid; or polychloronaphthalenes. The contractor relied heavily on "charcoal-foam" chromatography in his cleanup. In nearly every case, the spike material was not reported.

The contractor demonstrated that steam-distillation could be successfully employed to isolate polychlorinated dibenzo-p-dioxin from an aqueous matrix but is not acceptable for dioxin containing 5 or more chlorines in tissue.

This contract is in the process of being re-evaluated in view of the absence of the expected sample load and the poor reliability of the spiked sample analysis.

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 is enabling us to evaluate the reliability of methods which are being actively promoted for general usage.

LABORATORY OF MOLECULAR GENETICS

THE LABORATORY OF MOLECULAR GENETICS

Summary Statement

The Laboratory of Molecular Genetics (LMG) was formed in FY-79 during the course of an ongoing effort to reduce laboratories to more manageable sizes and to recruit experienced senior scientists into leadership positions within the NIEHS genetics community. The LMG is therefore closing out its first full year of independent existence, during which time a number of recently established programs have begun to reach their productive phases.

The LMG encompasses two very different but mutually supportive programs, one examining basic mutational mechanisms at the genetic and chemical levels and the other concentrating on testing large numbers of chemicals by the contract process and on developing and optimizing mutagen test systems. These programs are also complementary to the other Intramural Research Program laboratories in which genetic mechanisms constitute the central theme, namely the Laboratory of Animal Genetics (LAG) and the Laboratory of Biochemical Genetics (LBG). A major task of the LAG, for instance, is the study of eukaryotic gene structure and function, whose understanding is crucial for future LMG studies on biochemical mechanisms of mutation in eukaryotes.

The LMG has experienced two additions to its scientific staff this year. Dr. Barry Glickman arrived in mid-year from Leiden to take up an "Expert" position, and will found a group of several scientists to explore cellular aspects of mutagen specificity. Dr. Michael Volkert arrived late in the year from Berkeley to initiate a program concerned with inducible repair and mutagenic processes.

An important administrative change will take effect around the time of the FY-80/FY-81 transition. As part of an NIH-wide move to more clearly separate Intramural Research Program from National Toxicology Program functions, the contract testing functions of the mutagen testing program (the Environmental Mutagen Test Development Program) will be transferred from the LMG to the NTP. The Lawrence Livermore Laboratory program to explore mutagens generated during the course of food processing will also be transferred. These changes will relieve some of the administrative load in the LMG office and will allow some LMG scientists more time for their own research programs. At the same time, arrangements are being made to assure the continued stability and vigor of the research programs carried out by the EMTDP scientific staff.

THE MOLECULAR GENETICS PROGRAM

Heritable mutations severely affect several percent of humans, and more mildly affect another, larger fraction. Chemicals of both natural and artificial origin may exhibit mutagenic activities, and may contribute to both current and future human mutational burdens. While many mutagens can readily be detected by extant systems, such information is currently of limited

significance. First, ignorance of underlying mechanisms makes unclear the extent to which the same mutagens would affect humans. Second, the severity and qualitative nature of the human response depends upon generally unavailable information about mutagen specificity, that is, the classes of mutations produced by a particular mutagen. Third, intervention strategies suitable for situations in which a mutagen cannot readily be removed from the environment require very detailed understanding about how these mutagens act. To this end, the LMG maintains an active program to investigate mutagenic mechanisms in suitable model systems such as viruses, bacteria, yeasts and fruit flies. This group is composed of approximately equal numbers of geneticists and biochemists, although individual expertise frequently spans these two disciplines.

The group led by Dr. Akio Sugino concentrates on the underlying system upon which mutations impinge, namely DNA replication itself. Currently their work centers on two aspects of DNA replication, the enzymology of DNA topology in prokaryotic systems and the enzymology of DNA polymerization in eukaryotes. In the first project, the DNA gyrases (enzymes which determine the degree of twisting of intracellular DNA) have been characterized and compared from two very distinct bacteria, E. coli and Bacillus subtilis. In the second, some important advances have been achieved in our ability to understand and manipulate DNA replication in model Drosophila nuclear and mitochondrial and yeast nuclear systems. The origin of DNA replication has been located and cloned in the Drosophila mitochondrial system, and an in vitro DNA replication system has been established. Mutants of the nuclear DNA replicase (DNA polymerase alpha) have been obtained in cultured Drosophila cells. These constitute the first mutants to be discovered which specifically affect eukaryotic DNA replicases. Two classes of mutants have been isolated, both resistant to the drug aphidicolin: in one, the polymerase itself exhibits decreased sensitivity to the drug, while in the other, the polymerase is produced in sharply increased amounts within the cell. Both mutant types offer great promise as tools for the further investigation of enzymatic mechanisms of DNA replication in a higher eukaryote.

The group led by Dr. Lynn Ripley investigates mutagenic processes using the powerful bacteriophage T4 system. This system has, over the past three decades, provided a wealth of information about fundamental mutational mechanisms. Several projects are currently underway. (1) The process of spontaneous mutation is poorly understood, even though it presumably represents the major mutagenic process under many natural conditions. DNA within the cell is usually in a very active state, and is continuously bombarded by a variety of "natural" damages. These include attack by normal cellular metabolites and even by cellular heat, which is a mutagen. The mechanism by which premutational heat-induced lesions are converted by DNA metabolism into expressed mutations is being examined. In one series of measurements, the rate of cytosine deamination (a process which can lead to base pair substitutions) is being determined as a function of cytosine substituents (particularly 5-hydroxymethyl groups). In another, the fates of deaminated cytosine residues are being compared in host cells of varying competence with respect to their abilities to repair such damage. (2) The genetic determination of rates of frameshift mutagenesis is being probed as a function of the configuration of the phage DNA polymerase. It has already become clear

that the functional state of the polymerase strongly affects spontaneous rates of frameshift mutagenesis. (3) Accurate in vivo measurements have been achieved for the first time of the two steps in 2-aminopurine mutagenesis: misinsertion in place of adenine, and subsequent mispairing with cytosine. Both are under the control of the DNA polymerase and vary with its functional state, and the cytosine-mispairing step turns out to occur at an unexpectedly high rate. (4) The mode of action of the classical mutagen hydroxylamine has been re-examined. Predictions made from the conventional scheme for the mutagenic consequences of the reactions of hydroxylamine with cytosine have been tested, and in some instances the results have contradicted the chemical models. Chemical analyses are now underway to further explore this conflict.

Mr. Mark Conkling, a graduate student working under the occasional direction of the Chief of the LMG, is completing his studies on two aspects of mutagenesis in phage T4. The first involves an investigation of unneighborly base pair effects upon site-specific mutation rates. It has long been known that the mutational response exhibited by any particular gene is strongly dominated by the few most highly mutable sites within that gene, and that specific sites show a huge array of individual mutation rates. These rates have been shown to be determined not only by the target base pair itself, but also by its nearest neighbors on both sides, and to a limited extent by its penultimate nearest neighbors. Recently, however, a site in a T4 gene has been shown to exhibit a mutation rate which can be substantially altered by a base pair substitution within the same gene but, based upon genetic recombination frequencies, located some thirty base pairs away. The interaction between these two sites clearly cannot be electronic in nature, and a totally new mechanism of site-specific mutation-rate control is therefore implicated. Sequencing studies are now underway to determine the precise distance between these two sites, and perhaps also to provide hints as to the mechanism of the interaction. In a second project, the T4 post-replication mutational response to agents such as ultraviolet light, gamma rays and methyl methanesulfonate is being characterized. This response is conditioned by the coordinate action of several T4 genes, and is largely indifferent to the functional state of the host's own post-replication mutagenic response system. A number of mutants affecting the T4 system have been obtained and new methods have been worked out to characterize the role of these genes in the mutation process.

The group led by Dr. David Mace is investigating biochemical aspects of the mutation process. One project stems from our previous investigations of heat mutagenesis, a process which generates, among other mutations, transversions at guanine:cytosine base pairs. Our current model for this process implicates a guanine product, neoguanosine, in which the glycosidic bond has migrated from guanine N9 to the extracyclic N2 position (generating a structure apparently able to mispair with guanine itself as judged from model building). Methods have now been worked out to generate neoguanylic acid and deoxyneoguanylic acid from the corresponding GMP and dGMP precursors, and very preliminary results suggest that the generation of deoxyneoguanosine may be detectable in DNA itself. The next step in this investigation will be to determine the templating properties of such residues. Another major project focuses on the mechanisms by which DNA polymerases achieve their characteristically high fidelities during DNA replication. A useful probe of

polymerase fidelity is the base analogue N^6-methyladenine, presented as the deoxynucleotide triphosphate substrate in an in vitro polymerization reaction. The methyl group of this analogue can be oriented either cis (most of the time) or trans (occasionally) to the coding face of adenine; when cis it interfers with base pairing, but when trans it should be innocuous. The E. coli DNA polymerase I and the T4 DNA polymerase show rather different processing of the dNTP as a precursor of DNA chain elongation, and the further exploration of this difference may help to distinguish between alternative mechanisms for achieving fidelity during DNA replication.

The group led by Dr. Glickman is just getting underway. Initially they are investigating two aspects of mutagen specificity in E. coli. The first involves the putative mismatch repair system, a system which examines newly synthesized DNA for base mismatches (mutations), determines which is the "incorrect" (e.g., progeny) base on the basis of patterns of DNA methylation (newly synthesized DNA strands being undermethylated), and then excises the incorrect base. Mutations which block this system result in sharply increased spontaneous and induced mutation rates, and appear to fall into two classes: those which obliterate the methylation signalling system, and those which inactivate the hypothesized mismatch-excision system itself. The second project examines the role of cellular repair systems upon mutagen specificity. Using a recently developed system in which mutagen specificity can be assessed by the relatively rapid characterization of large numbers of mutations induced in the lacI gene, they have observed sharp differences in mutagen specificity with only relatively small differences in dose (in this case of methyl methanesulfonate). They have also demonstrated the induction of transversion mutations by MMS; transversions are particularly likely to produce deleterious amino acid substitutions in proteins.

THE MUTAGEN TESTING PROGRAM

This program has two major and complementary components. The first consists of research carried out by LMG personnel and directed towards the development and optimization of mutagen screening systems, the understanding of the mechanistic bases for the responses seen in these systems, and occasional trouble-shooting in relation to data generated in the contract testing component. The second aspect of the program consists of the development and supervision of contracts to achieve mutagenicity testing of fairly large numbers of chemicals or natural substances, including foods. The in-house research programs will be described first.

The group led by Dr. Errol Zeiger concentrates on the detection of mutagens by means of the Salmonella reversion test system, with particular attention to the crucial process of mammalian mutagen activation without which this bacterial system is impotent. Examples of ongoing systems optimization consist of studies of the effect of rodent age on the ability of microsomes to activate mutagens; efficacy of rabbit lung microsomes as activators (noting that the lung is a primary target of a large class of environmental toxicants); media effects on bacterial growth and response to mutagens; and the development of rapid strain verication methods for monitoring the continued genetic integrity of the (sometimes rather unstable) test bacteria.

Arsenic has been shown to decrease the mutagenicity of some chemicals in the Salmonella/microsome test, a result perhaps both of effects on mutagen activation by microsomes and of suppressed induction of the bacterial mutagenic response. The metabolic activation of polycyclic hydrocarbons such as benzo(a)pyrene by pathways other than the classical mixed function oxidases (for instance by prostaglandin synthetase) is being explored. A number of specific chemicals are also being screened for mutagenicity, including highly reactive industrial organic intermediates such as styrene oxides and cyclic hydrazides.

The group led by Dr. James Mason investigates mutagen-sensitive mutants of Drosophila melanogaster. Mutations which increase the sensitivity of an organism to chemical mutagens and irradiation are of dual importance: they provide powerful probes into underlying molecular mechanisms, and they provide strains with increased sensitivity which are useful in testing programs. The Salmonella/microsome system, for instance, depends heavily upon such mutants. A number of methyl methanesulfonate-sensitive mutants have now been isolated in Drosophila (12 on the X chromosome out of an estimated 60 in the entire genome), and have now been mapped by both recombinational and cytological methods. An unexpected result to date is that none of the tested mutants increase sponstaneous or alkylation-induced mutation rates as measured in the sex-linked recessive lethal test. Some of the mutants, however, reduce these rates.

The group led by Dr. Michael Resnick has been investigating the repair of DNA damage in the powerful model eukaryote Saccharomyces cerevisiae. An extensive set of repair-deficient mutants is available in this yeast, and methods have now been developed to detect repair following very low levels of DNA damage, a condition expected to be characteristic of environmental exposure conditions. Using DNA extraction methods which yield intact chromosome-long molecules, residual repair activity has been detected in some strains which were previously characterized as excision-defective at high doses. It has also been demonstrated that post-replication repair in yeast appears not to involve molecular recombination, in contrast to claims made for viruses and bacteria but consistent with claims made for mammalian cells. Finally, this group is exploring the effects upon the meiotic process of repair-deficient mutants, and the development of mutagen sensitivity during spore formation.

The contract testing program, EMTDP, has two interrelated purposes. The first is to test chemicals for mutagenicity, while the second is to test test systems themselves. The chemicals chosen for testing consist of those already in production, chemicals which are likely to receive lower priorities for industrial testing and governmental regulation than will those newly proposed for introduction onto the market. Within this rather large set of chemicals, priority is given to those which are produced in large amounts, for which there is significant human exposure, and for which ancillary information suggests the possibility of a potential for genotoxicity. There are perhaps 10,000 such suitable candidates for testing. With respect to the second aim of the program, there is little previous history to guide mutagen testing under realistic and rigorous conditions, that is, using highly defined protocols in a number of laboratories with constant quality

monitoring, predetermined data evaluation criteria and, most importantly, blind testing where the laboratory staff do not know the chemical under test and the interpretation of the data is carried out before the code is broken.

The first stage in EMTDP testing consists of the acquisition, storage and distribution of chemicals chosen for examination. This requires safety containment both in the storage facility and in the distribution process. The Chemical Respository contract also provides for the analysis of chemicals exhibiting mutagenic activity in order to determine which component is the actual mutagen; this is especially important in the case of industrial chemicals, where the "named" chemical may constitute as little as half of the mixture. The first and broadest testing component applies the Salmonella/ microsome test system, following a protocol designed to offer a currently optimum chance of detecting mutagenic activity if present. Another first-level testing component applies in vitro cytogenetic analysis; due to funding and laboratory limitations, however, not all chemicals subjected to microbial testing can be examined for cytogenetic effects. Therefore, chemicals which are negative in the microbial system are given highest priority for cytogenetic testing. At present the remaining component of the testing scheme utilizes Drosophila: chemicals which are positive in the previous systems are tested first for their abilities to produce gene mutations (via the sex-linked recessive lethal screen), and if positive there, are also tested for their abilities to produce heritable translocations.

It would be desirable to extend our testing in at least two major directions for which there exist good research bases but very little in the way of development towards standardized and validated protocols robust enough for the contracting mechanism. The first of these is specific-locus mutation in cultured mammalian somatic cells, for which there are several attractive candidate loci (HPRT, TK, OUA, APRT, etc.) but for which high interlaboratory reproducibility, optimal conditions for mutagen activation, and prior validation against a large set of chemicals is yet to be achieved. Currently, the EMTDP staff is exploring the feasibility of developmental contracts in this area. The second direction involves testing for aneuploidy, a major contributor to human genetic disease (the classical example is Down's Syndrome) for which there is as yet no well established screening system. Contracts are now being sought for the development of suitable aneuploidy-screening systems in both yeast and Drosophila.

The EMTDP generates large and rapidly increasing amounts of test data, which must be held available for evaluation by EMTDP personnel and release to the public via conventional outlets (including both publication and entry into the Environmental Mutagen Information Center data base). A data storage and retrieval system is currently under examination for adaptation to the specific needs of the EMTDP; it must be able to accept entries from the testing laboratories themselves, and to provide readout in the EMTDP headquarters. Furthermore, the data must be subjected to statistical analyses and evaluation by EMTDP personnel before the codes are broken, and to this end statistical programs are being developed suitable for each type of test system.

The final component of the mutagen testing program was developed independently of the EMTDP, and focuses not on industrial chemicals but on foods. A considerable number of foods, primarily of plant origin, have been shown to contain natural mutagens; and other foods have been shown to acquire mutagenic activities during preparation, primarily as a result of cooking processes. This result was sufficient to raise concerns, but not to provide a comprehensive survey, nor to evaluate the significance of such activities for humans. To this end, a contract with the Lawrence Livermore Laboratory provides for a survey of food consumption habits in North America, for surveys of food-borne mutagenic activities with particular attention to those generated by food processing, and for the chemical identification of such activities. Most recently this program has determined the conditions for the appearance of such activities in ground beef under realistic conditions of pan frying (without charing) and has purified one such activity over 100,000-fold. Another such activity, previously characterized, has been shown to be taken up rapidly from the mammalian intestine and to be retained in tissues for prolonged periods.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60052-04 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Environmental Mutagenesis Test Development Program (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:	D. Walters	Chemist	LEC	NIEHS
	B. Margolin	Statistician	BB	NIEHS
	J. Mason	Staff Fellow	LMG	NIEHS
	M. Resnick	Research Geneticist	LMG	NIEHS
	M. Rowley	Computer Systems Analyst	BB	NIEHS

COOPERATING UNITS (if any)

Laboratory of Environmental Chemistry, NIEHS and 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:
4.5	4.5	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 up to 1000 commercial and environmental chemicals per year for mutagenicity.

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 160 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 will test a total of 10 coded chemicals during the first 12 months of the contract.

Test System Validation: Protocols are under development 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 will standardize and validate a protocol using 10 to 15 open and coded chemicals, after which they will test a total of approximately 50 coded chemicals during the first 12 months of the contract.

Chemicals: A contract is ongoing at Radian Corporation, Austin, Texas, to maintain a chemical repository and analytical facility for the Environmental Mutagenesis Test Development Program. The repository is shipping coded chemicals to the Salmonella, Drosophila and cytogenetics contractors for mutagenicity testing. In addition they have begun analyses of chemicals found to give either a positive or questionable responses in Salmonella. A number of chemicals will be transferred from the NCI repository to the EMTDP repository for testing in Salmonella.

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. EMTDP is currently using an NCI contract at EG&G Mason Research Institute, Rockville, Maryland to capture Salmonella test data on a computer. This effort is being phased out and will be taken over by Bolt Beranek and Newman under the PROPHET contract. A study on the design and implementation of a data base management system for EMTDP has been done by Bolt Beranek and Newman. The study was reviewed by the Computer Systems Advisory Group who recommended that EMTDP utilize this system. Efforts are underway to add a task to the DRR contract for development and implementation of a data base management system (to be referred to as an Environmental Mutagenesis Information System (EMIS)). Under the EMIS system, Bolt Beranek and Newman will develop interactive data terminals to be placed in the testing laboratories for direct capture of test system data, and immediate statistical analysis of the data.

The system will also 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 and Drosophila. 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.

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 has not been evaluated for the potential to induced 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, An Environmental Mutagenesis Test Development Program, in IARC Publications in press.

Zeiger, E. (1979) Mammalian cell mutagenesis in a mutagen screening program. In Banbury Report No. 2, Mammalian Cell Mutagenesis: The Maturation of Test Systems. Ed. A. Hsie, J. P. O'Neill and V. K. McElheny, pg.145-153.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60077-03 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
Other:	D. Sherrick	Biological Lab. Tech.	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	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)

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.

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. To distinguish G:C-to-T:A from G:C-to-C:G transversions, a missense mutant having a serine (UCA) or a tyrosine (UAC) codon is needed.

MAJOR FINDINGS AND PROPOSED COURSE: In vivo measurement of mutation when C is substituted for glucosylated hydroxymethyl-C (glu-HMC) in heat-induced G:C to A:T transitions shows a lower frequency of detected mutations. The rate of mutation as a function of temperature or time at constant pH is decreased several-fold. The lower frequency of mutations may reflect one or both of two separate aspects of the DNA. One aspect is chemico-structural differences between C and glu-HMC DNA's. An example of a mechanism which might lower the frequency of mutation is a differential rate of deamination in C versus glu-HMC DNA at constant pH. In vitro measurements of the rates of deamination are proposed in conjunction with pH profiles of the reaction both in vivo and in vitro. The second aspect which might lower the frequency of mutation in C DNA involves possible differences in the metabolic processing of C and glu-HMC in the DNA or their mutagenic intermediates. If deaminated C (i.e. U) is indeed the mutagenic intermediate, accurate repair acting on U but not glu-HMU could give a reduced mutation frequency.

The host, E. coli, is known to possess an enzyme activity which removes uracil from DNA. The enzyme, uracil-N-glycosylase, depyrimidates uracil in DNA but does not depyrimidate HMU in DNA. E. coli mutants deficient in this enzyme are called ung. C-containing T4 DNA shows the same mutation frequency on ung$^-$ and ung$^+$ hosts, suggesting that this enzyme is not responsible for the lowered mutation frequency in C DNA; but these experiments are not definitive since residual enzyme activity may be present in the ung$^-$ mutant (less than 1% has been reported).

C-containing DNA is an unnatural form for T4 DNA and many differences between C and glu-HMC DNA may exist relating to the structure of the DNA in the phage head during heat treatment and to differential metabolism during growth of the phage.

The role that glucose alone may play in mutagenic differences between DNA's is just coming under investigation. The appropriate triple mutants are being constructed. These will then be heated to measure their reversion responses in the presence or absence of glucose.

The nature of mispairing of the heat-induced transition intermediate in vivo can now begin following upon the acquisition of adequate facilities (a warm room) and technical support (summer graduate student).

Previous genetic attempts to confidently discern the pathway of heat-induced transversion mutations (G:C to T:A versus G:C to C:G) have met with failure. I

propose to sequence an amber mutant and rUV74, a missense mutant, and their heat-induced revertants, to determine the major product of heat-induced transversion mutation. Appropriate rII mutants having C DNA have been constructed. Heat mutagenesis will be done to collect revertants in the C-DNA background. The C-DNA can be restricted and cloned on a lambda plasmid. Sequencing of these plasmids in the region of interest will be carried out collaboratively with sequencing already planned in a nearby region of the rII genome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Definition of the pathway of reversion is important because an important model for the mutagenic intermediate (neo-G) would be seriously challenged by the result that G:C to T:A as well as G:C to C:G was induced by heat.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60080-03 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:
0.3	0	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)

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.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60081-03 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

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:
0.5	0	0.5

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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. Finally the enzymology of the T4 misrepair system will be studied by genetic and biochemical approaches.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60082-03 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	S. D. Harris	Biological Aid	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	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)

This project investigates the possibility that deoxyneoguanosine residues in DNA (hyprothesized to be the G:C → C:G transversion pathway intermediate for heat mutagenesis in bacteriophage T4 can form a base pair with a deoxyguanosine residue on the opposing strand of a double helix. The formation of this novel base in polymeric and monomeric form is being studied. Its capacity to form a base pair with guanosine residues will be approached by both physical and biochemical methods.

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 substantial 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: Neoguanylic acid has been isolated in large amounts and is being chemically and physically characterized. The basic conditions for formation have been examined; these show its formation to be closely associated with acid-catalyzed depurination. Experiments designed to distinguish between an intermolecular and intramolecular reaction are also being conducted. A large batch of deoxyneoguanylic acid has been prepared for detailed analysis. Additionally, it is being used as starting material for the synthesis of labeled and unlabeled deoxynucleotide triphosphates. These can then be used for *in vitro* polymerization reactions and syntheses of polymers containing deoxyneoguanosine residues. Finally, for the detection of neoguanylic acid in DNA and RNA, enzymatic degradation and thin layer chromatographic analysis is being pursued. Preliminary results suggests the heat-mediated formation of deoxyneoguanosine in DNA, but thus far levels of radioactivity are very low. Thus, proof that the counts migrating with the neoguanylic acid marker are in fact deoxyguanylic acid has not yet been obtained. Protein-conjugated neoguanylic acid (later conjugated deoxynoguanylic acid) is being prepared for rabbit injection in hope of obtaining neoguanylic-specific antibodies. If deoxyguanylic acid residues can be shown to be formed in DNA, a search for repair systems will be undertaken.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60084-03 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Fidelity Mechanisms of Templated Polymerizing Enzymes

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

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:
0.6	0.6	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 goal of this project is to understand how templated nucleotide polymerizing enzymes achieve fidelity. Previous workers have concentrated on the 3' → 5' exonuclease associated with many DNA polymerases. However, the mechanisms by which the polymerizing step achieves accuracy have been largely ignored. To investigate the latter, the effect of polymerase substrate analogues on the fidelity of the polymerization reaction are being stuided primarily using T4 DNA polymerase as a model system. Modified nucleotide triphosphates are being utilized as probes of fidelity mechanisms. Initial efforts are focusing on rigorously testing the assumed importance of classical Watson-Crick base pairing by utilizing substrates with altered H-bond configurations. Kinetic steps will be probed with substrate analogues modified in the triphosphate moiety.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Established kinetic techniques are being used in the first phase of the experiments with substrate analogues. The analogues themselves are being prepared by a mixture of chemical and enzymatic synthesis methods; the later chiefly being used to convert the mono- or dinucleotide to the trinucleotide product. In addition, radioisotopically labeled substrate analogues are prepared chiefly by the Symmons chemical procedure or the polynucleotide kinase exchange reaction.

MAJOR FINDINGS AND PROPOSED COURSE: The insertion step of fidelity has long been believed to depend on direct hydrogen bonding of the substrate deoxynucleotide triphosphate with the template deoxynucleotide. While this is an elegant concept there are certain problems with this view. For example, a recent report on the utilization of N_6methyldATP in competition with dATP as a DNA polymerization substrate (both are inserted opposite T) showed that there was no discrimination against the modified substrate. Yet, other work by these authors showed that the methyl group at the N-6 position strongly prefers an orientation that would interfere with normal hydrogen bonding with a template thymidylate residue.

This was re-examined using the T4 DNA polymerase as a model system. In contrast to the observations with the E. coli enzyme, the T4 DNA polymerase does discriminate between the two substrates, utilizing the methylated base about seven times less well than the usual polymerization substrate, dATP. The reason for the striking difference between the two enzymes, and its implication for the role of base-pairing in polymerase fidelity is presently not clear, but experiments to test several hypotheses are underway.

While the above result with the T4 DNA polymerase is not inconsistent with the direct base-pairing model for fidelity, it does not confirm it. This is so because the methyl group can not only block one hydrogen bond with a template T, but it should also sterically hinder direct opposition of the two bases during insertion. To more directly probe the direct base-pairing model, other easily obtainable analogues of the normally utilized deoxynucleotide triphosphate substrates have been sought. There are a number of such possibilities, including: (a) inosine, a guanosine analogue forming only two hydrogen bonds with an opposing cytidine residue; (b) 2,6-diaminopurine, an adenine analogue forming three hydrogen bonds with an opposing thymidine residue instead of the usual two; (c) 2-aminopurine, an adenine analogue forming two hydrogen bonds with an opposing thymidine residue as does adenine itself but with the relative positions of the bonds altered (this analogue has already received considerable study but much of the interpretation depends on the model for the role of base pairing and exonuclease action that one adopts); and (d) 2-keto pyrimidine, a probable cytosine analogue which should form only two hydrogen bonds with a guanosine residue. In most cases the deoxyribonucleoside triphosphates of these analogues can be obtained without great difficulty (e.g., by a combination of chemical and enzymatic methods using commercially available nucleosides and nucleotides as starting materials).

As with the already synthesized and characterized N_6methyldATP, enzymatic polymerization of DNA with the novel substrates will be subjected to kinetic analysis. However, unlike N_6methyldATP, for which the methyl group probably interferes with AT base pairing sterically, the substrates listed above only have rearranged hydrogen bonding patterns. Such altered energies of hydrogen bonding could be expected to affect the strength of substrate-template interactions and thereby the kinetic parameters for them. These changes in hydrogen bonding are of the order of 1 kcal per mole; this should lead to a six to ten fold change in the apparent K_M for the analogue substrates. Such a change would be readily detectable, allowing a clear determination of the effects of hydrogen bonding on the initial selection of substrates by DNA polymerases. Already, kinetic analysis with deoxyinosine triphosphate has shown that it is used only slightly less well than deoxguanosine triphosphate despite its making only two hydrogen bonds with a template C. Currently, extensive experiments are underway with 2,6 diaminopurine deoxytriphosphate, and the preparation of purine deoxytriphosphate has begun.

In the future, additional determination of the analogues' base-pairing and templating characteristics will be sought by constructing DNA polymers containing these residues. This could be accomplished by untemplated synthesis using the calf-thymus terminal deoxynucleotidyl transferase, or by a more sophisticated approach using defined, natural, strand-purified DNA templates and restriction-nuclease generated fragments for site specific priming of DNA synthesis. Subsequent separation of the complimentary strands of the product would lead to a newly synthesized, substituted DNA strand which could in turn be used as a template.

Other possible mechanisms of polymerase fidelity will be probed also using analogues of deoxynucleotides. However, in this case the analogues should perturb neither the base-pairing properties nor that part of the molecule remaining after the insertion step is complete (i.e., the deoxymononucleotide portion). The later restriction should eliminate effects at any mononucleotide producing "proofreading" step, at least in principle. Fortunately, it is quite possible to perturb the β and γ positions of the phosphate chain in a variety of interesting ways.

It is hoped that we will be able to obtain such suitably modified substrates which function as substrates but not well. Poor functionality brought about by reduced substrate binding can be overcome by manipulation of concentration (again, at least in principle). On the other hand, poor functionality may also reflect disturbances of the active center of the polymerase. Such disturbances might then give rise to alterations in the overall fidelity of DNA insertion if fidelity-achieving kinetic mechanisms which are not base-pairing mediated are present. Of course, such substrates would have to be compared as pairs of competing nucleotides.

It is hypothesized that, in the absence of or in addition to substrate-template binding differences (and thereby discrimination between substrates), the actual kinetics of the polymerization catalysis may be different depending on

whether the correct or incorrect substrate is complexed. That is, the most efficient catalysis is achieved with the correct nucleotide triphosphate, while because of poor fit the "incorrect" triphosphate has less efficient catalysis. A general reduction of catalytic efficiency would less severely affect the already reduced catalysis of incorporation of the "incorrect" triphosphate. Such an "equalizing" effect would be equivalent to a loss in net selection for the correct nucleotide (i.e., an apparent loss of polymerase fidelity).

Finally, the recent research with N_6methyldATP has lead to a rather surprising result. Despite the expected discrimination (in comparison to dATP) by T4 DNA polymerase exonuclease against this modified substrate after insertion, there appears to be none during active DNA synthesis. On the other hand, direct measurements have shown the exonuclease to be capable of discrimination against the incorporated N_6methyldAMP; this exonuclease discrimination is also reflected in the proportinately higher turnover of the modified substrate during DNA synthesis even though the turnover discrimination (exonuclease proofreading?) does not affect the polymerases fidelity. Of course, this result would be the expected one if the vast majority of the turnover of triphosphate normally seen as a concomitant of in vitro DNA polymerization were an artefact of the particular properties of the enzyme. This is also under preliminary investigation to find a technique suitable to test the possibility.

In summary, using a series of easily obtainable deoxynucleotide triphosphate analogues in combination with detailed kinetic analysis of DNA polymerase reactions, it is proving possible to gain important new insights into the fidelity mechanisms of DNA polymerizing enzymes. With these tools, and the insights gained, new avenues of analysis will undoubtably open, not only for the prokaryotic polymerases thus far being studied, but also for later work with enzymes of higher organisms. Moreover, as the true nature of the mechanisms by which DNA polymerases achieve fidelity on their own, it will become possible to develop a real understanding of the ways in which other components of a multi-enzyme replication apparatus further increase fidelity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding the mechanisms used by DNA polymerases to accurately replicate the template during the polymerization step of DNA synthesis has largely been ignored until now. Such experiments are fundamental to an understanding of how the in vivo replication of DNA can be accomplished with sufficiently few errors to maintain a biologically acceptable spontaneous mutation rate. Using modified and unmodified triphosphates and comparing the relative fidelities of incorporation, it is hoped that insights can be obtained. Finally, since an understanding of the mutation process is one part of the overall goal of this Institute, learning how DNA polymerases achieve their remarkable accuracy will certainly be an important step in that direction.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60101-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Selection and 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
	B. Margolin	Mathematical Statistician	BB	NIEHS
	T. Clemmer	Computer Specialist	BB	NIEHS
	S. Kunitz	Head, Systems Design & Data Processing		NINCD
	R. Neff	Consultant		Harvard University
	F. Starmer	Professor		Duke University

COOPERATING UNITS (if any)

Biometry Branch, NIEHS, National Institute of Neurological and Communicative Disorders, Harvard University, and Duke University

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 consisting of Dr. Selma Kunitz (NINCDS, NIH), Dr. Frank Starmer (Department of Computer Science, Duke University) and Dr. Raymond Neff (School of Public Health, Harvard University) was formed to aid the EMTDP and Biometry Branch staffs in defining our 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. The EMTDP requested through DRR that BBN conduct a study to determine the software requirements of an Environmental Mutagenesis Information System (EMIS) and prepare a design document detailing the existing capabilities and enhancements required of PROPHET to build the EMIS.

The design document was completed in January, 1980 and evaluated by EMTDP and its consultants. It described several ongoing PROPHET activities which will lead to enhancements of PROPHET and fulfill the EMIS requirements, namely, to assist in the collection of EMTDP data from its contract laboratories, to provide a data base structure for the storage of this data and to provide PROPHET procedures for report generation and statistical analyses.

Work is presently underway 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. 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 several 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60102-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	J. Guthrie	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:
0.2	0.2	

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Salmonella tymphimurium strains TA-98, TA-100, TA-1535 are being used to detect ongoing chemicals of interest for mutagenicity. A series of cyclic hydrazides which are analogs of nitrosamines are being tested for mutagenicity for comparison with mutagenic and non-mutagenic nitrosamine counterparts. N-aminomorpholine. N-aminopiperidine, N-aminohomopiperidine, 1-amino-4-methylpiperazine, N-aminopyrrolidine, and 1,4-diaminopiperazine hydrate were all mutagenic, producing only base substitution mutations in the presence and absence of rat liver S-9. As a rule, more activity was seen in the absence of S-9.

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test procedure of Ames was used with some modification.

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. All the hydrazides except N-aminohomopiperidine showed a marked decrease in mutagenicity in the presence of the S-9 preparation. In the case of N-aminohomopiperidine the presence of the S-9 mixture produced no significant difference in TA-100, but the TA-1535 response appeared to increase. The mutagenicity results obtained with these hydrazides will be compared with mutagenicity results obtained with the corresponding nitrosamines.

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 the workers handling these chemicals. The responses of these chemicals may also give some indication as to whether or not they are intermediates in the in vivo and in vitro metabolism of nitrosamines to mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60103-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development of New and Improved Salmonella Test Methods

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
	I. Robertson	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	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)

Salmonella typhimurium tester strains are widely used for routine testing of unknown chemicals for mutagenic activity. This project is attempting to characterize such factors as the growth kinetics of the strains during the assay, effects of various growth media on the mutagenic response and on the kinetics of these responses.

PROJECT DESCRIPTION

METHODS EMPLOYED: During the course of a standard Salmonella plate test bacteria are recovered from the test plates and their growth rates charted to determine lag time and doubling time. The effects of various minimal media formulations and growth media are compared. The relationships between lag time, growth rate, and mutagenic response will be determined for a number of representative chemicals.

MAJOR FINDINGS AND PROPOSED COURSE: On Vogel-Bonner minimal E plates, the standard plates used in the Salmonella test, doubling times of 72-80 min were found with strain TA-1537 with a variable lag time of 1 to 4 hours. For comparison, on nutrient agar plates a lag time of 2-5 hours and a doubling time of 26-37 min was obtained. The lag time did not appear to be reduced if the overnight culture is grown in Vogel-Bonner medium supplemented with excess histidine instead of in nutrient broth. Use of a complex synthetic medium (Difco synthetic broth lacking histidine) resulted in an increase in doubling time and a reduction in numbers of spontaneous revertants. No growth was obtained using Difco histidine assay medium.

Results have also been obtained with Vogel-Bonner medium supplemented with a pool of amino acids at 20 µg/ml. Lag and doubling times have yet to be measured with this plating medium, but with strains TA-1537 and TA-100, using 9-aminoacridine and AF2 respectively, revertant colonies were obtained at 24 hours, compared to 40-48 hours with unsupplemented Vogel-Bonner medium. However, with strains TA-1535 and TA-98, using sodium azide and 4-nitro-o-phenylenediamine respectively, there was no difference, in the time of appearance of revertant colonies, between these two media. Further experiments are in progress to determine whether this effect is strain or chemical specific.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Strains of Salmonella typhimurium are widely used for routine testing of mutagens because of their sensitivity and ease of use. A further understanding of the strengths and deficiencies of these strains will enable us to better interpret Salmonella mutagenicity data. Also, it appears possible to make the Salmonella tests even more efficient and sensitive than they are now for detection of mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60105-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

B. Slatko, Department of Biology, Williams College, Williamstown, MA

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Laboratory of Molecular Genetics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

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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 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) Mutagen-sensitive mutants have been isolated and characterized as to their effects on DNA repair and are being tested for effects on mutation frequency; and (2) mutants which increase the spontaneous mutation frequency (mutators) will be isolated and characterized.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized mutants and chromosomal aberrations in Drosophila melanogaster are employed.

MAJOR FINDINGS AND PROPOSED COURSE: The X-linked mutagen-sensitive (mus) mutants have been tested for effects on the frequency sex-linked recessive lethals. None of the mutants increases the spontaneous mutation frequency, but two mutants at the same locus (mus-101) seem to decrease it. Mutants in the mus-101 gene are defective in post-replication repair of damaged DNA. Mutants with known defects in DNA repair have also been tested for effects on nitrogen-mustard-induced recessive lethals and heritable translocations. Again, mus-101 mutants show a decrease in both of these. Other mutants had no noticeable effect.

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. 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 deficiencies appear to be terminal on both cytological and genetic grounds. This mutant thus appears to be able to generate telomeres de novo. The mutant is being mapped so that a more thorough analysis can be performed.

Certain naturally occurring mutators increase recombination in both sexes, preferentially in centromeric regions. It is not clear whether this recombination occurs in euchromatin or in heterochromatin. To ask this question recombination will be monitored between two genes which are located in the centromeric heterochromatin of chromosome II with no euchromatin between them. It is also possible to monitor recombination between the heterochromatin on the X chromosome and the Y, which is entirely heterochromatic.

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.

PUBLICATIONS

Mason, J. M.: Spontaneous mutation frequencies in 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, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60106-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Staff Fellow	LMG	NIEHS
	N. N. Scobie	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

J. B. Boyd, Department of Genetics, University of California, Davis, CA

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Laboratory of Molecular Genetics

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

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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)

Mutagen-sensitive mutants defective in DNA repair mechanisms are collected in Drosophila melanogaster. The mutants are characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. The tests used in the initial characterization of these mutants include genetic and cytogenetic mapping, complementation analysis tests for sensitivity to unrelated mutagens, and tests for pleiotropic effects on related functions such as recombination. Some of these mutants may not be defective in DNA repair, but may affect metabolic (in)activation or permeability to chemical mutagens.

PHS-6040

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: Preliminary characterization of the mutagen-sensitive (mus) mutants has been completed. Most of the mutants so far have been isolated because of sensitivity to MMS. These comprise 12 complementation groups which map at random along the X chromosome. Except for two loci, the number of mutants at each locus fits a Poisson distribution with a mean of about 2. This suggests that all, or almost all, X-linked genes controlling sensitivity to MMS have been discovered. Since the X chromosome contains about 20 percent of the genome, this in turn suggests that there are approximately 60 of these MMS-sensitive loci in the Drosophila genome. In addition, there are likely to be genes which control sensitivity to other mutagens, but not MMS.

Cytological mapping of the mus mutants using duplications and deficiencies has been completed. The results agree with the genetic mapping of the mutants completed earlier. In addition, the deficiency mapping has helped to clarify the phenotypes of some of the mutants. One of the original mutagen sensitive chrmosomes (mus 107^{D1}) was shown to carry two mus mutations. In another case a mutation conferring mutagen-sensitivity (mus 106^{D1}) has been separated from a mutation conferring female sterility on the same chromosome.

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 which should clarify the allelic relationships among these mutants. Once these relationships are known we will have a better idea as to the number of pathways of post-replication repair that exist in Drosophila and how they are affected by caffeine.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60107-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	M. A. Conkling	Geneticist	LMG	NIEHS
OTHER:	A. Sugino	Visiting Scientist	LMG	NIEHS

COOPERATING UNITS (if any)

None

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Laboratory of Molecular Genetics

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

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0.3	0.1	0.2

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The bacteriophage T4 is a powerful tool in the analysis of basic mechanisms of mutagenesis. The genetics of the rII region have been extensively used and inferences of particular mutagenic pathways can be made. With molecular cloning and DNA sequencing techniques we can now confirm certain inferred pathways. Cloned sequences will also aid in the biochemical analysis of certain T4 functions involved in fidelity and repair. The genome of bacteriophage T4 will be cloned by a strain of phage recombinant DNA techniques. The primary cloning vector will be lambda. 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 randomly sheared to various lengths by sonication. Treatment with S1 nuclease will then produce blunt ends. Chemically synthesized EcoR1 linkers will be ligated to these ends using T4 DNA ligase. The fragments bearing EcoR1 linkers will be annealed and ligated to vectors and transfected into host cells. The progeny of the transfection should contain random clones of phage T4. Clones of interest will be selected by hybridization, complementation or marker rescue. These clones may then be sub-cloned on the plasmid pBR322. If the linker experiment does not yield the desired fragments we will restrict cytosine-containing T4 DNA. The restriction fragments will then be cloned following the above protocol.

MAJOR FINDINGS AND PROPOSED COURSE: We have had difficulty in obtaining good blunt end ligation of the EcoR1 linkers to the T4 DNA fragments. We have attributed this problem to improperly prepared T4 DNA substrates. We have initial shotguns of cytosine-containing T4 DNA but these have yet to be characterized.

Due to difficulties in cloning the T4 genome, we are developing a technique to sequence regions of the T4 rII genes without first cloning them. This technique will use an end-labled restriction fragment of the rII gene. This fragment will be annealed to T4 DNA and act as a primer for the dideoxy-sequencing method of Sanger.

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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60108-02 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mechanisms 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. Nakayama	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

Department of Biochemistry, the University of Chicago, Chicago, Illinois
Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH

Laboratory of Molecular Genetics

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1.25	1.25	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

DNA gyrase, which is involved in DNA replications, integrative recombination of phage λ DNA, transcription, and DNA repair, was purified from B. subtilis. The enzyme consists of the nalidixic acid (oxolinic acid) resistance and novobiocin (coumermycin A1) resistance gene products as does E. coli enzyme. In this year, the reactions catalyzed by B. subtilis enzyme have been compared with the reactions by E. coli enzyme. Moreover, heterogous DNA gyrases have been constructed biochemically and genetically to understand universal function of the enzyme.

PROJECT DESCRIPTION

METHODS EMPLOYED: Conventional column chromatographies and velocity sedimentation for purification of DNA gyrase. Agarose gel electrophoresis for assays of DNA gyrase activities. DNA-DNA and DNA-RNA hybridization (Southern and Northern blotting methods) and monoclonal antibody to characterize cloned genes.

MAJOR FINDINGS AND PROPOSED COURSE: E. coli DNA gyrase has many enzymatic activities, including DNA supercoiling, DNA untwisting, DNA catenation, DNA-dependent ATPase, and a DNA breakage reaction in the presence of nalidixic acid and SDS. In order to study the universality of DNA gyrase activities and structure, we have purified the activity from B. subtilis. B. subtilis is a more complex system than E. coli. Outgrowth from the spore state requires drastic changes of many enzyme activities, and it is widely speculated that such changes might be regulated by chromosome changes catalyzed by DNA gyrase and other enzymes. B. subtilis also has DNA gyrase activity and this is composed of at least two subunits, A and B (nalA and novA gene products, respectively), and each gene has been identified. These genes map close to the DNA replication origin and both genes' expression might be regulated with the initiation of DNA replication in B. subtilis. Recently, various novo^{ts} mutants from B. subtilis have been isolated (D. Dubunau, personal communication). In fact, DNA gyrases from these mutants are temperature-sensitive, proving that novA is a DNA gyrase gene. The DNA gyrase activities of B. subtilis are very similar to the E. coli DNA gyrase reactions. However, the DNA breakage reaction by the B. subtilis enzyme is about 20-fold weaker than that of the E. coli DNA gyrase. Nonetheless, the sites cleaved by the enzyme are the same.

Five-Kb DNA fragments which contain both the DNA replication origin and the nalidixic-acid-resistance gene (but not the novobiocin-resistance gene) have been isolated from EcoRI-treated B. subtilis DNA. Such a DNA fragment has been cloned in E. coli as well as in B. subtilis (K. F. Bott, manuscript in preparation). When this DNA fragment is put into E. coli, the nalidixic acid gene is expressed and complements with the E. coli nalidixic acid gene product to form a heterologous DNA gyrase, although the gene structure of the nalidixic acid protein is not homologous based on DNA-DNA hybridization tests. In order to extend this study, we will make an antibody against each subunit of the E. coli and B. subtilis DNA gyrases. Also, using such antibodies, we will study the biosynthesis of DNA gyrase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, particularly the importance of the tertiary structure of DNA, and will provide a corresponding understanding of mutagenic mechanisms.

PUBLICATIONS

Sugino, A., and Bott, K. F.: Bacillus subtilis deoxyribonucleic acid gyrase: J. Bacteriol. 141, 1331-1339 (1980).

Sugino, A., and Cozzarelli, N. R.: The intrinsic ATPase of DNA gyrase. J. Biol. Chem., in press (1980).

Sugino, A., Higgins, N. P., and Cozzarelli, N. R.: Covalent attachment of the DNA gyrase A protein to DNA. J. Biol. Chem. in press (1980).

ABSTRACTS

Cozzarelli, N. R., Brown, P. O. Kreuzer, K. N., Morrison, A., Otter, R. J., Gerrard, S. P., Sugino, A., Peebles, C. L., and Higgins, N. P.: DNA gyrase catalyzes the supercoiling, relaxation, and catenation of DNA by a sign inversion mechanism. ICN-UCLA Symposia, Molecular and Cellular Biology-Mechanistic Studies of DNA Replication and Genetic Recombination. J. Supramolecular Structure, supl. 4, p. 306 (1980).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60109-02 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
Others:	H. Kojo	Guest Worker	LMG	NIEHS
	K. Nakayama	Visiting Fellow	LMG	NIEHS
	B. Greenberg	Summer Graduate	LMG	NIEHS
	S. Ohnishi	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	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 DNA segment which contains the origin of mtDNA replication in Drosophila virilis has been cloned in E. coli and its nucleotide sequence has been determined. Using either native mtDNA or the chimeric DNA containing the origin of DNA replication, an in vitro mtDNA replication system has been developed from mitochondria isolated from Drosophila virilis embryos.

A mutant which possesses an altered DNA polymerase α has been isolated from Drosophila melanogaster Schneider cell line #2 using aphidicolin as a selective reagent.

An in vitro 2μm DNA replication system has been developed from a crude extract of the yeast Saccharomyces cerevisiae. The origin and direction of 2μm DNA replication have been determined both in vivo and in vitro.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard DNA cloning methods. Chemical and enzymatic DNA nucleotide sequencing methods. DNA-synthesizing crude-extract or semi-purified enzymes from isolated mitochondria of Drosophila embryos, or from wild type and cdc mutants of the yeast Saccharomyces cerevisiae. Agarose and polyacrylamide gel electrophoresis, sucrose density gradient sedimentation, and electron microscopy to analyze the products of in vitro DNA replication systems. Ethyl methanesulfonate mutagenesis using Drosophila melanogaster Schneider cell line #2 to isolate mutants which have an altered DNA polymerase α. Aphidicolin, a specific inhibitor for DNA polymerase α, for mutant selection. Conventional column chromatographies and velocity sedimentation centrifugation for purification of DNA polymerase α.

MAJOR FINDINGS AND PROPOSED COURSE:

A. Mitochondrial DNA Repliation in Drosophila

Mitochondrial DNA replication in Drosophila starts in the "A+T"-rich region of the circular molecular and proceeds unidirectionally. However, the precise location of initiation of DNA replication is still unknown.

(1) In order to localize the precise initiation point of mtDNA replication, Drosophila mtDNA treated with restriction endonuclease HindIII has been cloned in Escherichia coli using plasmid pBR322 DNA for a cloning vehicle, and the chimeric plasmid DNA containing the "A+T"-rich region of Drosophila mtDNA was identified. Using this plasmid DNA, we have constructed the detailed physical map of the "A+T"-rich region of mtDNA. Finally, the total nucleotide sequence of this DNA has been determined using either the Sanger enzymatic method or the Maxam-Gilbert chemical-modification method.

(2) The 6-7s first initiation product of mtDNA replication will be isolated from Drosophila embryos or cell cultures and labeled with [γ-^{32}P] ATP and polynucleotide kinase. The precise location of the intiation of this DNA will then be determined.

(3) At the same time, an in vitro mtDNA replication system has been developed using native and cloned mtDNA for probes. It mimics the in vivo system and is able to complement with exogenous proteins factors.

(4) The proteins which are required for the above in vitro DNA replication system will be isolated and purified from crude extracts from Drosophila mitochondria. Reconstitution of the DNA replication system will be attempted using these purified protein factors. During the reconstitution experiments, individual functions in DNA replication will be elucidated.

(5) Using this in vitro DNA replication system we will estimate error frequencies of the DNA polymerizing reactions.

B. Nuclear DNA Replication in Drosophila

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 generally 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 2μm DNA replication (A. Sugino, unpublished results).

First, this project focusses on the isolation of aphidicolin-resistant mutants and ts mutants from both established cell cultures of Drosophila and intact animals. In particular, we are going to use the same technique established by A. Greenleaf et al. who succeeded in isolating α-amanitin-resistant mutants from Drosophila melanogaster flies. If we succeed in isolating such mutants, we will purify the altered DNA polymerases from the mutants and characterize them extensively.

C. 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 DNA replication in yeast spheroplasts.

Drug-resistant mutants will be 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 2μm-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.

If we succeed in isolating the yeast DNA polymerase gene, we will characterize the gene and construct various mutant DNA polymerases using local mutagenesis techniques.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The in vitro DNA replication system from Drosophila mitochondria and the in vitro yeast 2μm DNA replication system are relatively simple model systems for complicated eukaryotic DNA replication. Using these systems, the proteins which play roles in DNA replication fidelity in eukaryotes may be identified. This in turn may help to understand some crucial aspects of mutation in eukaryotes.

PUBLICATION

Sugino, A.: Cloning of the replication origin from Drosophila virilis mitochondrial DNA. Biochem. Biophys. Res. Commun. 91, 1321-1329 (1979).

Sugino, A., Nakayama, K., and Kojo, H.: Structure and function of "A+T"-rich region in Drosophila mitochondrial DNAs. ICN-UCLA Symposia, Molecular and Cellular Biology - Mechanistic Studies of DNA Replication and Genetic Recombination. J. Supramolecular Structure, Supplement 4: 327 (1980).

Sugino, A., and Nakayama, K.: DNA polymerase α mutants from a Drosophila melanogaster cell line: to be submitted to Proc. Natl. Acad. Sci. (1980).

Kojo, H., and Sugino, A.: In vitro replication of the 2μm DNA of yeast Saccharomyces cerevisiae; The origin and direction of the DNA replication. in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60110-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	D. C. Mace	Senior Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

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1	1	0

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☐ (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: Data so far obtained on the reversion of rII mutants indicate there to be no large differences in the hydroxylamine-induced revertability of the rII genes with either HMC- or C-containing DNA. For example, both rUV7 and rNT88 revert about 1.5 times more readily with substituted C than with unsubstituted residues in their DNA. The presence of 5,6-dihydro-N^4-hydroxy-6-hydroxyaminocytosine, a product not formed with HMC, apparently has no effect on mutation in agreement with biochemical misincorporation assays by RNA polymerase. It evidently does not cause substantial lethality since both types of phage are equally sensitive to inactivation. O-methyl hydroxylamine, which is thought to react with pyrimidines in a similar manner to hydroxylamine, is also equally efficient in reverting r mutants with C- or HMC-containing DNA.

N-methyl hydroxylamine is unreactive toward HMC and only one product (N^4-methyl-N^4-hydroxycytosine) is formed upon reaction with C. Accordingly, rII mutants of T4 containing HMC in their DNA are not reverted by this methylated hydroxylamine. However, when these phages contain C DNA they are revertable, supporting the contention the N^4-methyl-N^4-hydroxycytosine is able to mispair.

Some of the biological data differ from those expected from available chemical data. For instance, the reactivity of HMC is very much less than C and yet phage containing either of these residues are equally mutable by hydroxylamine. There may well be substantial differences between the rates and products of reaction of hydroxylamine with bases and with DNA. These problems may be resolved by examining directly the products formed in T4 DNA.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60111-01 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies of 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
	G. Bayliss	Biological Aid "Q"	LMG	NIEHS

COOPERATING UNITS (if any)

None

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1.5	1.5	

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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)

DNA polymerase mutants of T4 are known which strongly influence the frequency of mutations occurring spontaneously or after mutagenic treatments. Both mutator and anti-mutator phenotypes have been discovered for transition base pair substitution mutations. Effects of these polymerases on frameshift mutations have been observed, but never systematically defined. The effects of mutationally altered polymerases on frameshift mutations might be expected to provide genetic clues as to the role of the polymerase in frameshift mechanisms of mutation and would identify mutant alleles of DNA polymerases which could be studied biochemically for altered properties which correlate to frameshift production.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 utilizing mutants of the rII genes to measure mutation rates and frequencies. We hope to characterize mutants which will be useful in distinguishing different types of frameshift mutations in these studies. (e.g. distinguishing addition frameshift mutations from deletion frameshift mutations, or frameshift mutations occurring in repeated sequences from those occurring in non-repeated sequences DNA sequencing of certain mutants and their revertants may be undertaken. Gene 43 mutants temperature sensitive for DNA synthesis are available. These mutants will be crossed into isogenic genetic backgrounds, paired with tester rII frameshifts and their effect on spontaneous and mutagen induced frameshift revertants measured. A search will be made for new gene 43 alleles which specifically enhance frameshift mutations.

MAJOR FINDINGS AND PROPOSED COURSE: Eighteen gene 43 temperature sensitive alleles have been screened for their ability to significantly alter the reversion frequency of r131 a frameshift in the rII A gene which is believed to be the result of a deletion of a single adenine in a run of 6 adenines in the DNA sequence. Ten were found to have significant effects upon the reversion at this frameshift site. Four show decreases in the spontaneous revertant frequency; six show increases. The four which show decreases are also antimutators for transition mutations. Among the six which showed increases, there are some alleles which have been shown to be mutators for base pair substitution mutations. However, not all alleles which have been shown to have mutator effects on base pair substitution mutations showed strong mutator effects at the r131 mutant site. Those eight alleles having little effect at the r131 site influenced the spontaneous revertant frequency at that site less than 3-fold. Additional sites are being tested with all the gene 43 alleles. We are looking for patterns by which we might determine whether polymerases show mechanistic or sequence specificity, thus the mutants we are using are primarily in sequenced regions of the rII genes.

Preliminary studies with a few alleles showing influence on spontaneous frameshifts have been done with proflavin mutagenesis. So far, alleles having mutator effects on spontaneous reversion of r131 also cause mutator effects upon proflavin-induced reversion. Similarly, there are antimutator effects for both types of reversion by antimutator alleles. Quantitatively there are differences however, analysis of the significance of this finding awaits further characterization of mutator and antimutator effects at additional rII sites.

We plan to look for frameshift mutator alleles by screening spontaneous and proflavin-induced revertants of double frameshift mutants after treatment of the phage with base pair substitution mutagens (hydroxylamine, or base analogues). If strong mutator alleles can be found by this technique, they will be genetically characterized. The genome of T4 is well-marked

genetically and mapping these mutator alleles to already known genes, or to locations for any new genes should be readily achieved. We are particularly interested to discover if there may be an important role for particular proteins of DNA replication to play in maintaining correct allignment of primers during DNA synthesis thereby preventing frameshift mutations.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60112-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

COOPERATING UNITS (if any)
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Dr. M. Radman, Free Univ. of Brussels, Brussels, Belgium

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	

CHECK 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.

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.

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 Hague, 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 Miroslav 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).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60113-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

COOPERATING UNITS (if any)
Laboratory of Molecular Genetics
Leiden State University
Leiden, 2333AL, The Netherlands

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) MAN 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.

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.

MAJOR FINDINGS AND PROPOSED COURSE: X-rays: Following a dose of 30 kR all possible base changes were observed without any obvious preference which suggests that there was no single major lesion responsible for X-ray mutagenesis. The specificity data showed that deamination was not a consequence of X-rays and that several sites within the lacI gene behaved differently than witnessed for spontaneous mutagenesis. The induction frequency for the total lacI$^-$ population differed from the frequency at which amber and ochre mutants were induced. The doubling dose for the total lacI$^-$ population was 11kR while that for the nonsense mutants was only 3 $\sim$ 4 kR.

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. A dose effect was observed, with the proportion of hotspots increasing with dose.

Akylating agents: Prelimiary experiments were carried out to determine the effect of cellular repair capacity and dose on the mutagenic and toxic effects of alkylating agents. The role of the recA, lexA, umuC, uvrA, uvrB, uvrC, recF, recL, uvrD and uvrE genes upon the repair of EMS and MMS induced damage was examined. In this way we were able to separate direct mutational effects and indirect effects; moreover, we found that a functional uvrA, uvrB, uvrC repair capacity was required for the excision of ethylation but not methylation base damage. Two recently discovered mutations, tak and

alk, both defective in the repair of alkylation damage, were also found to be hypermutable by EMS and MMS.

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 should 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. (submitted).

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Schaaper, R. and B. W. Glickman. UV-induced mutagenesis: a prerequisite for a pyrimidine dimer at the target site? in preparation (1980).

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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).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60114-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Activation of Polycyclic Hydrocarbons to Mutagens by Prostaglandin Synthetase

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
	J. Guthrie	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:
0.3	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)

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 mutagenicity of polycyclic hydrocarbons in the presence of prostaglandin synthetase. Benzo(a)pyrene, benzanthracene and their non-mutagenic metabolites will be tested for mutagenicity using this system after activation by prostaglandin synthetase.

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.

MAJOR FINDINGS AND PROPOSED COURSE: The detergent commonly used for the isolation and solubilization of prostaglandin (PG) synthetase is toxic to Salmonella in the concentrations normally used for metabolic studies. Additionally, the combination of arachidonic acid + 0.25 M sucrose is toxic to the cells. These problems have been circumvented by using 0.15 M KCl in place of the sucrose. By filtering the PG-synthetase preparation prior to further purification steps, the need for detergent is eliminated. With these modifications studies on mutagenicity of benzo(a)pyrene and benzanthracene and their metabolites using prostaglandin synthetase and arachidonic acid can proceed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most current research on the activation of pro-carcinogens to carcinogens is with the mixed function oxidases. There is some indication that PG-synthetase may also serve as an activation system in vivo. If this is the case, it presents many new questions on the metabolism of toxicants. Our work will help to further elucidate the role of PG-synthetase in the activation of toxicants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60115-01 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

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
Other:	D. Pagano	Research Microbiologist	LMG	NIEHS
	B. Yagen	Visiting Scientist	LP	NIEHS

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Molecular Genetics

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.55	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)

Salmonella typhimurium tester strain TA-100 is being used in a study of the reaction rates of styrene oxide isomers. It has been noted previously that the (+) and (-) styrene oxide isomers showed different rates of conjugation with glutathione. To date, we have shown that the (-) isomer produces the same level of mutagenic response as the racemic mixture (1:1) over a timed course of exposure.

The addition of uninduced rat liver soluble enzymes (cytosol) and glutathione to the preincubation mix, decreases the mutagenic response for both the (-) isomer and the racemic mixture. Additional testing performed on styrene glycol and styrene-glutathione conjugate at concentrations up to 10 x the styrene oxide levels, showed no mutagenic or toxic responses.

PROJECT DESCRIPTION

METHODS EMPLOYED: The Ames Salmonella/microsome test is being used with modification of the pre-incubation procedure to allow timed samples to be examined.

MAJOR FINDINGS AND PROPOSED COURSE: The (-) isomer and racemic mixture of styrene oxides show similar mutagenicities when tested in timed pre-incubation exposure. The addition of glutathione does not decrease the mutagenicity unless cytosol from uninduced rat liver S-9 is added to the pre-incubation mix.

Further work will involve varying the cytosol concentration over shorter pre-incubation times. Also, a (+) isomer of styrene oxide may be included in these studies.

Additionally, conjugates of styrene oxide will be tested for mutagenicity.

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 will also determine whether the conjugates of styrene which appear in the urine retain any biological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60116-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
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SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.05	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)

Sterile filter paper discs are impregnated with crystal violet, nitrofurantoin, sodium azide, 4-nitro-o-phenylenediamine and 9-aminoacridine. Another disc, ampicillin, is commercially available. Each Salmonella tester strain, TA-100, TA-98, TA-1535, TA-1537, TA-1538 responds differently to each chemical. Selective use of the discs on a single plate provide a broad spectrum of reactions which are diagnostic for specific strains and can be used to confirm the genotypes of the strains.

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 one month. 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, which are normally 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 and negative control system and allow for the positive identification of each strain used at the time of its use.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60117-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
A Study of Tris(2,3 dibromo propyl phosphate) and its Metabolites 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
Other:	D. Pagano	Research Microbiologist	LMG	NIEHS
	A. Nomeir	Visiting Scientist	LPK	NIEHS

COOPERATING UNITS (if any)

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.40	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)

The Ames Salmonella/microsome strains TA-100 and TA-1535 are being used to test for the mutagenicity of Tris, 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 Tris. All positive responses required Aroclor-induced rat liver S-9.

PROJECT DESCRIPTION

METHODS: Standard Salmonella/microsome plate test procedure was used.

MAJOR FINDINGS AND PROPOSED COURSE: Two metabolites, $C_7H_{12}Br_3O_4P$ and $C_7H_{13}Br_4O_4P$, gave mutagenic responses equivalent to those obtained from Tris; one metabolite, $C_7B_{11}Br_2O_4P$, gave approximately one-half the Tris response, while a $C_5H_{11}Br_2O_4PP$ compound was about one-fourth the level. All required metabolic activation with S-9 which implies that these substances are not the ultimate mutagens formed from Tris. Further work is planned using other metabolites and chemical compounds of similar structure.

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 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 metabolites will provide an understanding of the mechanism of action of Tris, 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60118-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Effects of Arsenic Exposure on Liver Activation of Mutagens in Salmonella Test Systems

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
	J. Guthrie	Microbiologist	LMG NIEHS
	C. Squibb	Postdoctoral Fellow, Biochemistry	LOFT NIEHS
	B. Fowler	Research Biologist	LOFT NIEHS

COOPERATING UNITS (if any)
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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	

CHECK 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 of long-term exposure of arsenic on the ability of hepatic enzymes to convert pro-mutagens to mutagens were tested in both rats and mice. The S-9 fractions isolated from these animals were tested in the Salmonella activation test (strain TA-98). The following compounds were used: 2-acetylaminofluorene, 2-aminoanthracene, 3-methylcholanthrene and benzo(a)pyrene. The results indicate that the mutagenicity of some, but not all, chemicals is depressed in arsenic-exposed animals.

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard Salmonella plate test procedure of Ames was used with some modification.

Both mice and rats were administered arsenic in their water for six weeks. After six weeks they were sacrificed, livers of animals in the same group were pooled and S-9 was prepared from these livers. The arsenic content of S-9 from each group was assayed.

MAJOR FINDINGS AND PROPOSED COURSE: Depression of the enzymatic conversion of pro-mutagens to mutagens can occur following exposure to arsenic through drinking water. This depression is generally slight and does not encompass all pro-mutagens. In mice this depression occurs with 2-aminoanthracene and 3-methylcholanthrene and in rats it is found with 2-acetylaminofluorene.

Future work will involve the addition of arsenic to S-9 from unexposed animals to elucidate the relationship between arsenic concentrations and enzyme depression; inclusion of Aflatoxin B_1, in the list of chemicals tested; retesting using another strain, TA-100.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the United States there exist certain areas in which higher-than-recommended levels of arsenic are found in the drinking water. Demonstration of altered liver activities in arsenic-exposed animals indicates that ingestion of arsenic in the diet may affect the ability to metabolize toxicants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60119-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Senior Staff Fellow	EBB NIEHS (NTP:NCI)
	E. Zeiger	Supervisory Microbiologist	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

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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)

Salmonella typhimurium tester strains are of use in examining the metabolic activation of chemical carcinogens using mutation as the endpoint. This project will determine 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, both S_{16} supernatant and microsomal fractions, from both young and old female Long-Evans rats will be incorporated in a standard Salmonella plate test.

MAJOR FINDINGS AND PROPOSED COURSE: This project has just been initiated. The tissue preparations have been made. The chemical doses and tissue homogenate levels giving a linear dose response are being determined.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60120-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
	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 Molecular Genetics

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	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)

Salmonella typhimurium tester strains are of use in examining the metabolic activation of chemical carcinogens 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. 10-76)

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 will be further characterized using monooxygenase systems, reconstituted from purified components, in the Salmonella test. Such characterization will include the effects of antibodies to and inhibitors of specific forms of cytochrome P450.

MAJOR FINDINGS AND PROPOSED COURSE: Work to date is preliminary and has been aimed at identifying which carcinogens of interest are mutagenic to Salmonella using rabbit lung activation.

Acetylaminofluorene, 2-aminofluorene, 2-aminoanthracene and aflatoxin B_1, are mutagenic to Salmonella strain TA-98 when incorporated with a 16,000 x g supernatant of lung homogenate. However, the two polycyclic hydrocarbons benzo(a)pyrene and 3-methylcholanthrene gave a weak, poorly-reproducible response.

If the activities are standardized to per nmol of total cytochrome P450, the S_{16} supernatants of the lung homogenate give 3X more activity with 2-aminoanthracene, 20X with 2-aminofluorene and comparable activity with acetylaminofluorene and aflatoxin B_1 compared to similar hepatic preparations tested at the same time.

Dimethylnitrosamine was inactive in a pre-incubation assay with the his G46 strain of Salmonella but this lack of activity may be due to the low cytochrome-P450 content of the lung S_{16} supernatant.

NADPH-dependent metabolism of acetylaminofluorene and aflatoxin B_1 to mutagenic products were also catalyzed by pulmonary microsomal (160,000 x g) fractions. Under these conditions (0.6 nmoles cytochrome P450/ml activation mix) benzo(a)pyrene and 3-methylcholanthrene appear inactive. As a point of comparison, although the major form of pulmonary and hepatic cytochrome-P450 are different, the mutagenic activity of acetylaminofluorene was similar with preparations from either tissue, but the activity of aflatoxin B_1 with hepatic microsomes was greater. Benzo(a)pyrene and 3-methylcholanthrene were also inactive with the hepatic preparations.

Other carcinogens are in the process of being tested. Once mutagenicity is established the metabolic activation will be further characterized using the monoxygenase systems reconstituted from purified components.

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.

As these systems are known to contain many forms of cytochrome P450 with different substrate specificities, it may be possible to relate the organ susceptibility to specific forms of the cytochrome or to significant differences in the activities of activating or detoxifying enzymes such as gluathione transferase or epoxide hydratase. For this reason a detailed comparison of the ability of these systems to metabolize carcinogens to mutagenic products, and an analysis of the roles of individual pulmonary and hepatic cytochromes is being made.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60121-01 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

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	LMG NIEHS
	M. Smalls	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:
0.25	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 both the NIEHS PDP11 computer and the PROPHET System.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A non-interactive program for analyzing sucrose gradient results has been obtained from Dr. Jay Donniger, NCI and Dr. Richard Setlow, Brookhaven National Laboratory for modifications.

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.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60122-01 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

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)

Dr. Brian Cox, Botany Department, Oxford University, England

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.15	0.30

CHECK 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 yeast is under the control of more than 20 genetic loci. Damage (primarily pyrimidine dimers) produced by UV-light can be repaired through three pathways. We have developed a method for identifying and analyzing the repair of pyrimidine dimers by these pathways after extremely low doses corresponding to only 1-2 dimers per chromosome. Chromosomal size DNA obtained through gentle lysis of cells is treated with Micrococcus luteus extract which recognizes and nicks the DNA at pyrimidine dimers. The DNA is subsequently analyzed using sucrose gradient techniques. This method has enabled us to identify low levels of repair (leaky) in previously identified repair deficient mutants and to demonstrate that post-replication repair in yeast does not occur via a recombinational mechanism.

PHS-6040
(Rev. 10-75)

METHODS EMPLOYED: Various mutants are genetically manipulated and grown using techniques standard for handling yeast. Analysis of repair involves the exposure of yeast to UV-irradiation, followed by post-irradiation intervals for repair. The DNA is radioactively labeled prior to irradiation with ^{3}H uracil and post-irradiation synthesis is identified using ^{14}C uracil. 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.

MAJOR FINDINGS AND PROPOSED COURSE: We have developed a lysis and sucrose gradient technique which enables the identification of full-size chromosomal DNA in yeast. This method, which involves a gentle lysis of cells and is not affected by the post-irradiation fragility of cells, allows for the detection of less than 1-2 single-strand breaks or pyrimidine dimers per chromosome. Using this method, we have been able to examine excision repair and post-replication repair after low doses of UV (2-4 J/m^2). Some strains, which had previously been identified as being excision defective after high doses, did, in fact, exhibit excision after low doses. Using complete and also "leaky" excision defective mutants, we have demonstrated that the post-replication repair process does not involve molecular recombination. These results are comparable to those for mammalian cells. Because of the ability to genetically manipulate yeast, these observations will enhance our understanding of the nature of the post-replication repair process and its role in mutagenesis. Using these techniques we also plan to investigate the repair of damage due to other types of agents, particularly low levels of ionizing radiation.

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. Since these pathways are involved in mutagenesis, this work will further our understanding of the basic mechanism of mutation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60123-01 LMG

PERIOD COVERED

October 1, 1979 to September 30, 1980

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
	S. Foster	Biologist	LMG	NIEHS
	M. Smalls	Biological Aid	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:
1.7	0.4	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Using the yeast Saccharomyces cerevisiae we are examining the importance of DNA repair in normal meiosis and following exposure to DNA damaging agents during meiosis. Mutants in the three genetically defined UV and X-ray repair pathways are being used: rad 1 (excision repair), rad 52 (X-ray repair) and rad 6 (mutational repair). The rad 6 and rad 52 mutants do not prevent a normal round of meiotic DNA synthesis; however, recombination is abolished. No meiotic spore products are formed in rad 6 strains, whereas with rad 52 the spores are inviable. The rad 52 strains accumulate single-strand breaks or gaps in parental DNA during meiosis, whereas in RAD$^+$ there is a transitory appearance of breaks. We conclude that the RAD 52 gene product which is required for normal meiosis is involved with recombination and the breaks may be intermediate in this process. Further experiments are underway to test this. We are also probing UV-induced mutations and postreplication processes during meiosis and comparing them to their mitotic counterpart using excision-defective strains. These studies will enable us to determine if there are repair mechanisms unique to meiosis and the effectiveness of mutagenic agents administered during meiosis.

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 radioactivity-labeled cells are examined for the appearance of breaks using sucrose gradient techniques and for the repair of UV-induced pyrimidine dimers (after UV treatment). 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 and their protective action against DNA damaging agents administered during meiosis. At the genetic level we have established that the gene products required for the repair of X-ray damage in mitotic cells are necessary during normal meiosis. Mutations in the rad 6 gene, 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 rad 52 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.

Previously we established that rad 52 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 have now shown that during meiosis, the rad 52 mutants accumulate single-strand breaks (or gaps) in parental DNA during and after DNA synthesis and these breaks are not restituted. In the RAD$^+$ strains there is an indication of short-lived breaks (this requires further investigation). These experiments were performed using techniques which we have developed for the quantitative recovery of chromosomal size DNA during the various stages of meiosis using alkaline and neutral sucrose gradient techniques. These results are the first report of any mutation affecting meiosis which leads to specific changes during meiosis other than gross alterations in DNA synthesis. Using these and other mutants we are continuing to probe the nature of DNA changes during meiosis with emphasis on the importance of DNA repair mechanisms.

We have also begun to examine the nature of DNA repair processes during meiosis when cells are challenged by external agents. The strains which are developed specifically for these and the above studies exhibit synchronous meiosis. Using UV as a probe, we have established that the sensitivity of meiotic cells does not change appreciably during meiosis until the formation of meiotic spore products, when the cells become more sensitive. We are beginning to evaluate the effects of UV and X-rays (and later other mutagens) on the completion of meiosis and the induction of mutation when the cells are exposed at various stages of meiosis. Coincident with the studies we will be measuring the extent of repair in the wild type and various repair-deficient strains.

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, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60124-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

A contract is being prepared for the development of an aneuploidy test system in the yeast Saccharomyces cerevisiae. It is expected that the successful contractor(s) will be able to develop a microbial system which will enable the rapid screening of agents that induce aenuploidy during meiotic development and mitotic growth. In addition, the system(s) will be able to screen and compare the effects of agents in terms of the induction of recombination, 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.

PROJECT DESCRIPTION

METHODS EMPLOYED: The development of this contract and its award will proceed through standard contract procedures.

MAJOR FINDINGS AND PROPOSED COURSE: The concept of this contract has received approval in a concept review process by a group of scientific peers. By the end of FY 80 or early FY 81 it is expected that the contract(s) will be awarded.

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. 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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60125-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Development of Drosophila Aneuploidy Test

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

PI: J. M. Mason Staff 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:
0.1	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 purpose of this work is to award a contract which will be used 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.

FORM-2040

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Test procedures using Drosophila melanogaster will be developed for use in a test for aneuploidy induced by industrial and environmental chemicals. Strains will be produced which can be used to detect aneuploidy i.e., the gain or loss of a whole chromosome. Optimal conditions for several variables will be established and a final testing protocol will be developed.

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 in vitro microbial tests 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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60126-01 LMG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Chemical Repository for Mutagenicity Screening

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

PI:	D. B. Walters	Chemist	LEC	NIEHS
Other:	E. Zeiger	Supervisory 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:
0.6	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 purpose of this Contract is to establish and maintain a repository for approximately 3000 compounds which are to be screened for genetic toxicity. Available physical - chemical, toxicological and safety information is provided on all compounds, as well as provisions for procurement, storage, distribution, chemical analysis for stability, purification, synthesis and reference archiving. Capabilities for sophisticated chemical trace analysis also exist.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The contractor receives chemicals which are to be tested for mutagenicity by bioassay laboratories under contract. 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 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 approximately 30 samples per month including controls and replicates. An estimated 10% of test compounds are analyzed for trace chemical impurities.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 222 test compounds and 25 controls have been selected for FY 80 testing. 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. The computer inventory system is being modified to accommodate current and anticipated changes necessary to serve increased NTP requirements. Satisfactory survivability tests have been completed and approximately 193 aliquots have been shipped to 13 test laboratories. Approximately 35 chemicals are being subjected to chemical analysis. Three chemicals are being synthesized for NTP testing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a comprehensive testing system for mutagenesis (as well as other NTP 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 chemical assay as well as sophisticated, complete, chemical tract impurity analysis. These requirements are necessary to support *in vitro* and *in vivo* testing.

PUBLICATIONS

1. Walters, D.B. (editor): Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Vol. I, Ann Arbor Science, Ann Arbor, Michigan, April, 1980.

2. Walters, D.B. (editor): Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Vol. II, Ann Arbor Science, Ann Arbor, Michigan, April, 1980.

3. Walters, D.B., McKinney, J.D., Norstrom, A., Dewitt, N.: Control of Potential Carcinogenic, Mutagenic and Toxic Chemicals via a Protocol Concept and a Chemistry Containment Laboratory, in Safe Handling of Chemical Carcinogens, Mutagens, Teratogens, and Highly Toxic Substances, Vol. I, Chapter 1, Ann Arbor Science, Ann Arbor, Michigan, 1980.

4. Hunt, C.L., Walters, D.B. and Zeiger, E.: Approaches for Safe Handling Procedures and Design of a High Hazard Laboratory for Life Scientists, in Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Vol. I, Chapter 2, Ann Arbor Science, Ann Arbor, Michigan, 1980.

5. Harless, J., Baxter, K.E., Keith, L.H. and Walters, D.B.: Design and Operation of a Hazardous Materials Laboratory, in Safe Handling of Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Vol. I, Chapter 4, Ann Arbor Science, Ann Arbor, Michigan, 1980.

6. Ward, J.T., Williams, C.H., Walbach, C.D., Keith, L.H. and Walters, D.B.: Transportation of Materials from Radian Corporation's Hazardous Materials Laboratory, in Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances, Vol. I., Chapter 5, Ann Arbor Science, Ann Arbor, Michigan, 1980.

7. Moore, J.A., Huff, J.E., Walters, D.B.: Overview of the National Toxicology Program in Environmental Health Chemistry: The Chemistry of Environmental Agents as Potential Human Hazards, Ann Arbor Science, Ann Arbor, Michigan, 1980 (in press).

8. 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, Ann Arbor Science, Ann Arbor, Michigan, 1981 (in press).

9. 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, Ann Arbor Science, Ann Arbor, Michigan, 1981 (in press).

10. 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).

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 (NIEHS): Douglas B. Walters, Ph.D., Chemist, LEC

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $110,731

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for 3000-4000 compounds which are to be screened for mutagenicity. 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 bioassay laboratories under contract. 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 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 laboratroy 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 are being synthesized for special NTP needs.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 222 test compounds and 25 controls have been selected for FY 80 testing. For FY 80 200 test compounds and 25 controls have been received by the repository. 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 is nearing completion on implementing a computerized inventory and data handling system. Satisfactory survivability tests have been completed and approximately 193 aliquots have been shipped to 13 test laboratories. Approximately 35 chemicals are being 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.

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 (NIEHS): Errol Zeiger, Ph.D., Supervisory Microbiologist
Environmental Mutagenesis Test Development Program, LMG

DATE CONTRACT INITIATED: 1. December 22, 1978
2. December 29, 1978
3. February 1, 1979

CURRENT ANNUAL LEVEL: 1. $43,103
2. $51,989
3. $80,530

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 will be selected for chemical analysis and further testing in Drosophila and for cytogenetic effects in cultured mammalian (Chinese Hamster ovary) cells.

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 will be incubated with the tester strains in suspension prior to addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation will be provided by liver S-9 preparations from Aroclor 1254-induced Sprague-Dawley rats and Syrian Hamsters. All chemicals will be tested blind at 5 doses, in triplicate, in each Salmonella strain. Also, all chemicals will be retested at least 2 weeks following the first test. 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 160 test samples to date; 147 of these test samples have been decoded. It is anticipated that an additional 100 chemicals will be tested this calendar year, and that 300 chemicals will be tested per year in future years.

Manuscripts are currently being written to present results of the initial chemicals in the open scientific literature.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These contracts will allow the NIEHS 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 the highest 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., Staff Fellow
Errol Zeiger, Ph.D., Supervisory Microbiologist
Environmental Mutagenesis Test Development
Program, LMG

DATE CONTRACT INITIATED: 1. September 28, 1979
2. September 28, 1979
3. September 28, 1979

CURRENT ANNUAL LEVEL: 1. $ 96,746
2. $110,860
3. $108,148

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 30 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: The three laboratories have demonstrated that they all get the same mutagenicity results using coded control chemicals. Testing aspects of these contracts have just gotten underway and complete results are not yet available. It is expected, however, that testing will proceed rapidly.

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 pro-mutagens by means of an enzyme system similar to the mammalian activation system. Chemicals found to be mutagenic, therefore, will be given the highest priority for chronic toxicological and carcinogenesis testing by the National Toxicology Program.

1. COLUMBIA UNIVERSITY - New York, New York
(NO1-ES-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, Research Geneticist, Division of Toxicology, FDA

DATE CONTRACT INITIATED: 1. September 29, 1979
2. September 29, 1979

CURRENT ANNUAL LEVEL: 1. $174,519
2. $165,003

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to develop and validate a protocol for testing of 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 exact protocol to be used is currently being developed 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: Two control chemicals have been tested openly by both laboratories after a number of discussions on the design of the protocol. Results in the two laboratories show excellent comparability. Testing is now underway with other known mutagens that require S-9 metabolic activation for activity. If good comparability is achieved here, both laboratories will test ten coded known samples. If the laboratories show good agreement with each other and with the expected results, they will begin testing unknowns.

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.

LAWRENCE LIVERMORE LABORATORY - Livermore, California
(222Y01-ES-80038)

TITLE: Survey of Cooked Foods for Mutagenic Activities

CONTRACTOR'S PROJECT DIRECTOR: M. L. Mendelsohn, Ph.D.

PROJECT OFFICER (NIEHS): John W. Drake, Ph.D., Chief, LMG

DATE CONTRACT INITIATED: September 22, 1978

CURRENT ANNUAL LEVEL: $400,000

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to survey the food consumption habits of the general population of the U.S.A., to screen appropriate foods for mutagenic activities using a modified tier approach with particular emphasis on mutagenic activities introduced by food processing procedures (especially cooking), to determine the chemical identities of such mutagens and their mechanisms of formation, to determine their potencies in mammalian systems and to design practical avoidance procedures.

METHODS EMPLOYED: The survey aspects of this program are subcontracted to the Department of Nutrition of the University of California, Berkeley, who also carry out food acquisition and preparation. The initial mutagenicity assay will consist of the Ames battery, supplemented by sister-chromatid exchange and cultured mammalian cell specific-locus mutation assays. Chemical fractionation of complex mixtures in order to isolate mutagenic components will employ a variety of chromatographic methods.

MAJOR FINDINGS AND PROPOSED COURSE: The appearance of mutagenic activity in ground beef has been confirmed during cooking under realistic pan-frying conditions (200°C) without charring, and the major activity has been purified over 100,000-fold and characterized as an organic base. It induces frameshift mutations in Salmonella tester strains and requires metabolic activation of a somewhat unusual type. A similar activity has been produced at 90°C after boiling and dehydration of beef stock and is formed from small molecular weight precursors (probably amino acids or small peptides). The kinetics of mutagen formation have been determined as functions of cooking temperature and cooking surface, with indications that mutation formation depends upon rate of heat transfer and degree of dehydration. The North American diet has been assessed for major sources of protein intake and predominant cooking methods, providing testing priorities for the future. A radiolabeled mutagen of the type produced by protein pyrolysis has been synthesized and shown to be rapidly absorbed intestinally with prolonged tissue retention.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The now clearly established presence of mutagenic activities introduced into foods by the process of cooking raises major questions concerning the

distribution of such activities in typical North American diets and the risks which they may - or may not - pose to human health. A broad and comprehensive attack is needed, one which will range from survey through analysis to assessment of risk and finally to prevention of genotoxic effects.

LABORATORY OF ORGAN FUNCTION AND TOXICOLOGY

LABORATORY OF ORGAN FUNCTION AND TOXICOLOGY
Summary Statement--FY-1980

ORGAN FUNCTION AND TOXICOLOGY

A new laboratory, Laboratory of Organ Function and Toxicology (LOFT), has evolved from the former Laboratory of Environmental Toxicology. It consists of researchers from a variety of scientific disciplines: toxicology, physiology, pharmacology, pathology, biochemistry and endocrinology. This laboratory provides a central focus at the NIEHS for ongoing investigation of the effects of environmental agents on renal, gastrointestinal, and hepatic function and proposed investigations on the cardiovascular and immune systems. It plans and conducts studies to elucidate the mechanisms by which environmental agents alter normal functions of these organ systems including the role of metabolism, endocrine factors, and toxicant-receptor interactions, and it plans and conducts studies to identify sensitive indicators of organ specific toxicity useful in the early detection and prediction of toxicity in experimental animals and humans. The Laboratory collaborates with epidemiologists in developing methods for extrapolation of data from experimental animal models to estimation of human risks from exposure to environmental agents.

HEPATIC AND ENDOCRINE WORKGROUP

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. A multidisciplinary approach is utilized to identify sensitive biochemical indicators of hepatotoxicity that might have clinical value; elucidate mechanisms of hepatotoxicity; understand the regulation of hepatic protein synthesis including the role of the hypothalmic-pituitary-gonadal axis; and characterize the role of endocrine action in the regulation of hepatic function in control and 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. In contrast to the uterus, 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 and preliminary data indicate that this protein may facilitate nuclear translocation of estrogen-receptor complex. 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.

Research efforts are in progress to elucidate the mechanisms of perinatal imprinting of hepatic metabolism by hormones and hormonally-active xenobiotics such as the PCBs. Imprinting is defined by the ability of neonatal hormones to permanently and irreversibly program for the sex differentiation of hepatic function. Although the process is initiated in the neonatal brain, differentiation is expressed postpubertally and is regulated by the pituitary-hypothalamic axis. Newborn rats treated with DES or TP exhibit no significant changes in the prepubertal activities of several hepatic enzymes (5α-reductase, 16α-hydroxylase, histidase, UDP glucuronyltransferase, ethylmorphine demethylase, monamine oxidase, etc.). However, the subsequent adult animals exhibit masculinized or feminized levels of enzyme activity and these effects appear to be permanent and irreversible. A series of experiments utilizing ectopic pituitaries and hypophysectomized rats suggest that imprinting of enzyme activity is mediated through the pituitary-hypothalamic axis during a critical period of early development. Although, imprinting of hepatic enzyme synthesis might have a common mechanism, different regulatory models can be constructed for different groups of enzymes. Hepatic protein profiles are being mapped by 2-D electrophoresis in an attempt to further understand the sex differentiation of hepatic function in control and treated animals. Further experiments show that sensitivity to the toxic effects (liver and testes) of cadmium are permanently altered qualitatively and quantitatively by newborn exposure to hormonally-active xenobiotics. These changes in hepatic response to cadmium appear to be related to altered synthesis of cadmium-senstive proteins and enzymes.

RENAL AND INTRACELLULAR WORKGROUP

Scientists in this workgroup study basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in vivo. The goal is to apply this information to designing rapid and effective test procedures for predicting toxicity in man with specific emphasis on the kidney.

Principal efforts are oriented towards investigation of the mechanisms involved in producing the earliest signs of subcellular damage by both quantitative electron microscopy, biochemical, and physiological measurements. Particular emphasis is placed on understanding the mecahnisms by which environmentally important chemicals interact with these 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. Previous studies from this group have shown that renal uptake

of cadmium bound to cadmium metallothionein (Cd-MT) is mediated via the proximal tubule cell lysosome system with subsequent degradation of the metalloprotein to release Cd^{+2} ion. The mechanism by which this process causes marked necrosis of proximal tubule cells is under current investigation. A second area of intense interest involves the mechanisms behind toxicant damage to mitochondrial structure-function relationships and the role of this phenomenon in important mitochondria-mediated clinical entities such as Reye's Syndrome. Toxic agents like arsenic have been shown to alter mitochondrial membrane structure and synthesis with attendant compromise of biochemical processes dependent on the physical integrity of this organelle including the synthesis of heme and urea. Further studies are in progress to delineate the molecular basis of these structural changes and their relationship to biochemical mitochondrial dysfunction.

Another area of effort involves combined use of ultrastructural and morphometric and biochemical techniques to identify early effects of heavy metals and other environmental agents on mammalian tissues so that early warning 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 agents for various periods. In current studies, researchers are examining the toxicity of arsenic, cadmium, lead, methyl, and inorganic mercury. Results of their experiments indicate that specific morphological and biochemical response profiles can be established which differentiate various metals and other agents in our multi-element environment. Studies are currently in progress to use these profiles to develop test procedures that will permit identification of preclinical exposure to specific environmental substances in human populations by measurement of circulating metabolites such as porphyrins or excreted classes of specific low molecular weight proteins.

GASTROINTESTINAL WORKGROUP

This workgroup focuses on developing approaches to study problems of intestinal toxicology and function. Of particular interest are areas that represent unknowns in the field of intestinal absorption and metabolism, and in the responses related to oral exposure to toxins. Current examples of these areas are the preferential use of energy sources by the isolated intestinal absorptive cells, the regulation of intestinal glucuronidation and sulfation, and the organ specificity of colon carcinogens. The mechanics of these processes are examined necessarily at the cellular, subcellular, and molecular levels.

We are interested in studying: (1) the effects of antioxidants on colonic NAD-linked dehydrogenases and the role of colon dehydrogenases in the metabolism of methylazoxymethanol and aldehyde formation; (2) the interactions between acrylamide and intestinal processes as well as intestinal metabolism of acrylamide; (3) possible effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on intestinal protein and lipoprotein synthesis; (4) the employment of absorptive function tests for monitoring oral exposures to toxins; (5) the effects of T-2 toxin from Fusarium on intestinal function and tumor formation; and (6) the effects of altered redox state on the transport and metabolism of a p-nitrophenol analogue by the isolated intestinal absorptive cells.

A better understanding of the basic principles of normal intestinal functions 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 malabsorption. The possible interactions of ingested substances both in natural and metabolized forms are rapidly increasing 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 of hydrazine, 1,2-dimethylhydrazine, polychlorinated-biphenyls, arsenic, and acrylamide as well as to in vitro effects of substituted toluenes and aromatic aldehydes.

COLLABORATIVE RESEARCH

Laboratory scientists are Principal Investigators in the wide variety of research contract efforts. These collaborative efforts are logical extensions of the Laboratory's intramural programs concerned with target organ toxicities.

LOFT and LRDT investigators are collaborating with Raltech of Madison, Wisconsin to search for the transplacental toxicity of selected environmental chemicals. This contract fulfills a critical need to identify chemicals or classes of chemicals that might adversely affect organ function following gestational exposure. Chemicals currently under study include several PCB and PBB isomers, DDE, polycyclic hydrocarbons, and a series of mycotoxins. LOFT scientists are collaborating with BB and local hospitals in evaluating the relationship of PCB levels to altered placental function emphasizing the ability of placental microsomes to metabolically activate carcinogens.

The LOFT and Brigham Young University are attempting to identify clinically-useful biochemical indicators of developmental toxicity. The approach is to first investigate the ontogeny of key enzyme systems following treatment with known teratogens.

Collaboration between LOFT and NOAA scientists is investigating the physiological effects of arsenic, cadmium, and copper on marine shellfish. This effort is directed at elucidating the mechanisms by which marine species accumulate toxic metals in comparison with mammals. A cadmium-binding protein has been isolated from shellfish which appears to play an important role in the bioaccumulation of this toxicant by commercially important species. The toxic actions of cadmium and copper on gill respiration are being examined in relation to induction of this protein. Another collaborative effort between LOFT and FDA scientists concerns dietary interactions between Pb, Cd, and As. Results from these studies indicate that concomitant exposure to Pb and Cd reduces tissue concentrations of Pb but not the biological effects of Pb as assessed by increased urinary porphyrin excretion. The relationship between the intracellular distribution of metals such as lead and biological activity in multi-element exposure situations is under further study.

The role of hormone receptors in xenobiotic-induced alterations of hormone responsiveness is being studied in collaboration with the UNC Department of Biochemistry. This project should yield information important to understanding the mechanism of action of endocrine-mediated hepatotoxicity. In a related project, scientists in LOFT and Roosevelt Hospital, New York, are attempting to utilize hepatic histidase as a marker indicative of perinatal imprinting of hepatic metabolism.

The LOFT is concerned with the role of the redox state in the regulation of the relative rates of glucuronidation and sulfation of xenobiotics within the intestinal absorptive cells. A chemist at the Research Triangle Institute has collaborated in advancing this research interest by developing an enzyme-isotopic-chromatographic method to measure UDP-GA levels in tissue extracts.

Another collaborative effort involves the effects of T-2 toxin from Fusarium which was isolated by a Chemist from the Hebrew University, on the gastrointestinal tract after acute exposures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70120-08 LOFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

PI:	G. W. Lucier	Acting Chief	LOFT
Others:	E. Lui	Visiting Fellow	LOFT
	O. McDaniel	Bio Lab Tech	LOFT
	W. Powell-Jones	Visiting Associate	LOFT
	C. Lamartiniere	Senior Staff Fellow	LOFT

COOPERATING UNITS (if any)
Laboratory of Pharmacology

R. Philpot	Research Chemist	LP
S. Slaughter	Staff Fellow	LP

LAB/BRANCH
Laboratory of Organ Function and Toxicology

SECTION
Hepatic and Endocrine

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

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 hepatotoxic responses. The rat is used as the experimental model. The initial focus has involved sex-differentiation of the hepatotoxic effects of 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.

OBJECTIVES AND METHODS

I. Describe sex differentiation of 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 the HMOS. 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 HMOS 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 and ethionine as hepatotoxic chemicals; cadmium and ethionine alter HMOS in a sex-dependent manner. Endocrine influences described in Part III 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 are 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.

V. Assess hepatotoxicity and the role of HMOS in in vitro systems. Attempts will be made to identify early indicators of hepatotoxicity.

MAJOR FINDINGS

Sex-related differences in the hepatic microsomal ethylmorphine N-demethylation activities were observed only in control mature rats; specific activities in males were 4-fold higher than in females. Administration of TP or 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 at the pituitary-hypothalamic level during a critical period of neonatal development by androgens.

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 demonstrate the critical neonatal neonatal period for the ability of methadone to feminize the HMOS.

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.

PROPOSED COURSE

Future studies will focus on the: (1) nature of 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 pepipheral endocrine systems on the regulation of hepatic enzymes.

The mechanisms of the sex-dependent response to the hepatotoxic actions of cadmium will be studied by: (1) evaluating metallothionein 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. Collaborative efforts are being established with Dr. Jerry Rice (NIH, Head, Perinatal Carcinogenesis Section) to determine the capacity of endocrine status during a critical period of early development to program susceptibility to hepatocarcinogens by irreversibly imprinting function of the HMOS.

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. Since 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 cirtical 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 hepatotoxicity.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70132-01 LOFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Gastrointestinal Function and Toxicology

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:
3.5	1.0	0.0

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Our focus is on developing approaches to study problems of gastrointestinal toxicology and function. Of particular interest are areas that represent unknowns in the field of intestinal absorption and metabolism, and in the responses related to oral exposure to toxins. Current examples of these areas are the preferential use of energy sources by the isolated intestinal absorptive cells, the regulation of intestinal glucuronidation and sulfation, and the organ specificity of colon carcinogens. The mechanics of these processes are examined necessarily at the cellular, subcellular and molecular levels.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

Method 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 uniquely by us to monitor intestinal processes in laboratory animals include: 1. measurement of in vitro protein biosynthesis in the subcellular fractions of isolated intestinal tip and crypt cells; 2. separation of intracellular contents from suspensions of monodispersed absorptive cells; 3. subcellular fractionation of isolated intestinal cells; 4. assay of intestinal cell glutamine- and glutamate-metabolizing enzymes and gluconeogenic enzymes; and 5. absorptive function tests.

Of special interest is the method we devised for measuring cellular levels of UDP-glucuronic acid (UDPGA). Our method involves the conversion of UDP-GA to PNP-GA in the presence of liver microsomes and ^{14}C-p-nitrophenol (^{14}C-PNP). The ^{14}C-PNP and ^{14}C-PNP-GA are recovered, separated and quantitated by HPLC. This procedure allows us to detect levels of UDP-GA in tissue extracts that are below the limits of the widely-used spectrophometric assay. This method will be used to study the relationship between metabolically altered UDP-GA levels and the relative rates of glucuronidation and sulfation in isolated intestinal absorptive cells.

Major Findings:

Colon-specific carcinogenesis:

The synthetic compounds 1,2-dimethylhydrazine and azoxymethane, both chemically related to the naturally occurring carcinogen cycasin, have proved to be of great value in their reliable and specific ability to produce colon tumors in several rodent species. With animal models, it becomes possible to study modifying influences on the initiations 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. The incidence of tumor formation in the treated animals was 78.6% as compared to 0% for the control animals. All of the tumors were located (1 to 3 per rat) in the colon with the exception of one in the Zymbal's gland of the ear and one in the small intestine. This is the lowest single oral dose of 1,2-dimethylhydrazine reported to produce colon tumors.

Our interest in colon energy metabolism led us to a study in which pregnant Fischer rats were given this same level of 1,2-dimethylhydrazine (35 mg/kg) orally on the 18 day of gestation and several energy-related enzymes were monitored at various stages during development in the colon. The enzymes of interest were the glycerol 3-phosphate dehydrogenases, malate dehydrogenase and L-glutamate-oxaloacetate transaminase which are the constituent enzymes of the glycerol 3-phosphate and malate/aspartate substrate cycles for the transport reducing equivalents. However, none of these colon enzymes nor marker enzymes of the colon brush border were significantly altered in the offspring after prenatal exposure to 1,2-dimethylhydrazine. This experiment will be repeated at higher dose levels of 1,2-dimethylhydrazine.

In addition, pregnant hamsters were exposed by intubation to a single oral dose of hydrazine hydrate (260 mg/kg) or 1,2 dimethylhydrazine dihydrochloride (150 mg/kg) as a neutralized solution on Day 12 of gestation (4 days before birth). Similarly, doses of methylazoxymethanol acetate (340 and 85 mg/kg) were given and found to be lethal to the dams within a 24- to 48-hr period. Groups of these animals (N=3) were sacrificed 1 or 2 days before birth and 3 or 4, 10, 17, 24 or 25, and 53 or 60 days after birth to evaluate the development of intestinal brush border enzymes, lactase, sucrase and alkaline phosphatase, as compared to the enzyme development in control animals. The results indicate that exposure to hydrazine diminished neonatal lactase activity, elevated postnatal and young adult sucrase activity, and elevated the neonatal and postnatal activity of alkaline phosphatase. In contrast, after prenatal exposure to 1,2-dimethylhydrazine, the postnatal level of sucrase activity and all levels of alkaline phosphatase were elevated. There was no significant effect of 1,2-dimethylhydrazine on the development of lactase activity. A screen for teratogenic effects revealed no incidence of cleft palate formation in the offspring. While there are no apparent teratogenic events, biochemical alterations reveal more subtle effects of these environmental toxins on this developing organ system. A similar study revealed the effects of hydrazine and 1,2-dimethylhydrazine on the plasma membrane enzyme associated with transport, Na, K-stimulated adenosnine 5'-triphosphatase. The results indicate that intestinal Na, K-ATPase is responsive to prenatal enhancement and that fetal intestinal cells are capable of reacting to a stimulation by hydrazine and 1,2-dimethylhydrazine. Whereas altered enzyme activities are being utilized currently as indexes of toxicity in general toxicology studies, we are not aware of any results, other than our own, that monitors altered enzyme activities in a specific organ after prenatal exposure to a carcinogen specific for that organ.

Feeding studies involving cycasin have indicated that pretreatment with an antioxidant n-butylated hydroxyanisole (BHA) provides protection against the carcinogenic effect of the 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 methylazoxymethanol a metabolite obtained from cycasin and from 1,2-dimethylhydrazine. Our initial in vitro studies on the inhibition of purified alcohol dehydrogenase and NAD-linked glycerol 3-phosphate dehydrogenase by n-butylated hydroxy-anisole, phenol and toluene indicate a more generalized inhibition of the compounds on NAD-linked dehydrogenases. Currently, we are examining the properties of several dehydrogenases from male Fischer rat colon tissue.

Energy Metabolism and Regulation of Metabolic Pathways

In addition to our studies involving colon energy metabolism and 1,2-dimethylhydrazine metabolism, we have a more general concern with the role of the redox state in the regulation of metabolic pathways and possible alterations to this regulation by environmental toxins. In particular, we have shown that prolonged oral exposure to arsenic results in inhibition of the key mitochondrial enzyme pyruvate dehydrogenase and in the altered mitochondrial redox state which may diminish the availability of other NAD-linked dehydrogenase substrates and products and, thus, alter the subcellular exchange of key metabolites.

Previous studies involving the effects of ethanol on the relative rates of glucuronidation and sulfation in liver tissue suggested that the redox state may be a factor. Our interest in the mechanism by which changes in NAD/NADH effect changes in cellular UDP-GA levels through the NAD-linked dehydrogenase and thus the availability of UDP-GA for the glucuronidation of hydroxylated intermediates has been limited by the ability to measure cellular changes in UDP-GA levels. We have developed recently an enzymatic-isotopic-chromatographic method to measure cellular levels of UDPGA which is applicable to numerous kinds of tissues.

Our interest in isolated intestinal absorptive cell energy metabolism has led to the examination of the preferential use of glutamine as an energy source. Intestinal cells utilize glutamine more rapidly and preferentially to glucose or glutamate. The level and subcellular location of the glutamine- and glutamate-metabolizing enzymes and the cellular levels of glutamine and glutamate were measured. Although membrane permeation may be a factor, our results suggest that the glutamine is readily converted to glutamate within the mitochondria by a phosphate-dependent glutaminase and thus is diluted only by mitochondrial glutamate before entering the tricarboxylic acid cycle as α-ketoglutarate. In addition, the levels of both phosphoenolpyruvate carboxykinase and pyruvate kinase in the intestinal mucosa are sufficient to provide pyruvate and thus acetyl-CoA for the tricarboxylic acid cycle. The other enzymes required for gluconeogenesis from glutamine, glucose-6-phosphatase and fructose 1,6 diphosphatase, are also present in the intestinal mucosa.

Mitochondrial Functions

Our investigations with isolated mitochondria have led to an evaluation of the in vitro effects of substituted toluenes. 3,5-Dinitro-4-chloro-α,α,α-trifluorotoluene (DNCTT) is an intermediate in the synthesis of the herbicide trifluralin and is structurally similar to the known uncouplers of oxidative phosphorylation 2,4-dinitrophenol (DNP) and pentachlorophenol. DNCTT (1mM) completely inhibited mitochondrial respiration with succinate (1.2mM) as substrate and in the presence of adenosine 5'-diphosphate (0.1mM). Although DNP (0.2mM) uncoupled state 4 respiration in the absence of DNCTT, DNP (0.2mM) did not uncouple DNCTT inhibited respiration. In addition, DNCTT (2.5mM) inhibited significantly the activity of the mitochondrial enzyme succinic dehydrogenase, but had no effect on the activity of cytochrome c oxidase and Mg-stimulated adenosine 5'-triphosphatase. The inhibitory effects of DNCTT were greater than those of eight other substituted toluenes examined. From the results of these studies, it appears that the effects of DNCTT on respiratory activity are a consequence of the inhibition of the mitochondrial enzyme succinate dehydrogenase. In view of the marked effects of DNCTT on mitochondrial activity, further work on the precise mechanism of action and chronic toxicity of DNCTT are warranted.

Collaboration

The recent report from the behavorial toxicologists that oral exposure to acrylamide has diet-dependent effects on behavior has led to the consideration of acrylamide for our absorptive function tests. Although acrylamide is a neurotoxin, we chose to examine several intestinal enzymes in offspring from pregnant Fischer rats exposed by gavage to 20 mg acrylamide/kg/day on ten successive days (days 6 to 17 of gestation). Measurements of maternal intestinal enzyme

levels showed no significant change, there were no overt signs of maternal toxicity, and the litter sizes, number and sex ratio of pups were uneffected by the dose of acrylamide given. However, the results of this cross-fostering experiment indicate effects of both a transplacental and lactational passage of acrylamide or a metabolite that alters intestinal enzyme activities in the pups.

In addition, we have studied the effects of polychlorinated biphenyls (PCBs) on the development of several intestinal and serum marker enzymes. Three congeners 4-monochloro (1-CB); 3,4-3',4'-tetrachloro(4-CB) and 2,4,5-2',4',5'-hexachloro (6-CB) biphenyls were administered orally to pregnant rats on days 8, 11, 13, 15 and 18 of gestation. 1-CB and 6-CB were administered at doses of 30mg/kg/day and 4-CB was administered at doses of 3 mg/kg/day. The altered levels of the serum and intestinal marker enzymes may be related to intestinal accumulation of PCB glucuronides in the late fetus. Altered enzyme levels are considered indexes of toxicity and may be useful in monitoring early and long-term changes in the normal processes of cellular metabolism and in identifying sites of toxic insult. These observations provide further insight into the toxic properties of PCBs on developing systems.

Our interest in mitochondrial function has also led to an examination of a metabolite of xylene tolualdehyde and other structurally-related aldehydes. Initial results indicate substrate-specific effects of benzaldehyde on mitochondrial respiration. Benzaldehyde also alters several mitochondrial enzymes including Mg-stimulated adenosine 5'-triphosphatase and cytochrome c oxidase.

Proposed Course:

We plan to concentrate our efforts in fiscal year 1981 on follow-up studies based on some of our current projects. In particular, we are interested in studying (1) the effects of antioxidants on colonic NAD-linked dehydrogenases and the role of colon dehydrogenases in the metabolism of methylazoxymethanol and aldehyde formation; (2) the interactions between acrylamide and intestinal processes as well as intestinal metabolism of acrylamide; (3) possible effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on intestinal protein and lipoprotein synthesis; (4) the employment of absorptive function tests for monitoring oral exposures to toxins; (5) the effects of T-2 toxin from Fusarium on intestinal function and tumor formation; and (6) the effects of altered redox state on the transport and metabolism of a p-nitrophenol analogue by the isolated intestinal absorptive cells.

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 in absorption and metabolism. In addition, this better understanding of normal function may lead to better methods for the detection of dysfunction and malabsorption. The possible interactions of ingested substances both in natural and metabolized forms are rapidly increasing 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 of hydrazine, 1,2-dimethylhydrazine, polychlorinated-biphenyls, arsenic, and acrylamide as well as to in vitro effects of substituted toluenes and aromatic aldehydes.

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3. Fowler, B. A., Woods, J. S. and Schiller, C. M. Studies of hepatic mitochondrial structure and function I. Morphometric and biochemical evaluation of in vivo perturbation by arsenate. Lab. Invest. 41: 313-320 (1979).

4. Schiller, C. M. and Walden, R. Processes of transport and absorption in the developing infant intestine. Rev. of Infec. Dis. 1: 674-680 (1979).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70135-03 LOFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Effect of Hormones and Xenobiotics on the Regulation of Perinatal Enzyme 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)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Our research efforts are directed toward investigating the effect and mode of action of hormones and xenobiotics on the regulation of enzyme patterns during perinatal development. Particular emphasis has been placed on the postnatal development of hepatic enzymes in the rat following perinatal exposure to gonadal hormones [testosterone propionate (TP) or estradial-17-β(E_2)] and hormonally active xenobiotics [polychlorinated biphenyl (PCB), diethylstilbestrol (DES), dichloro-diphenyl-trichloro-ethane (DDT) and methoxychlor]. We have investigated the altered ontogeny and sex differentiation of several hepatic enzyme markers following prenatal exposure to the above mentioned chemical and the results of our studies on the role of the hypothalamic-pituitary-gonadal axis on the regulation of some of these enzymes. These studies have demonstrated that the regulation of hepatic enzyme development is different for different groups of enzymes and that the critical period for neonatal imprinting of enzyme activity is not the same for all enzymes. Further studies are investigating altered sex differentiation of hepatic enzymes by mapping protein profiles on 2-D gels.

P. S-6040
(Rev. 10-76)

Methods Employed: Studies are carried out using birth-dated CD-stock random bred albino rats. Neonatal castrations (day 1) are performed on rats hypothermally anesthesized on ice. Neonatal exposure to testosterone priopionate diethylstilbestrol (DES) β-estradiol (E_2) or dihydrotestosterone (DHT) is carried out by subcutaneous injections of 1.45 μmoles of the hormone or 0.02 ml propylene glycol (PG) on day 2 post-partum. Methoxychlor or o,p'-DDT is administered neonatally on days 2, 4, 6 post-partum using sesamine oil as vehicle at a concentration of 1 mg or 3 mg/animal. Transplantation of pituitaries was done by placing pituitaries of age-matched rats under the kidney capsule of hypophysectomized rats anesthesized with metaphane.

Major Findings and Proposed Course: We have chosen 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 glutatione S-transferase may however be controlled by a hypothalamic inhibiting factor.

UDP-glucuronyltransferase. The hepatic microsomal enzyme, UDP-glucuronyltransferase, undergoes a complex developmental pattern in which enzyme activity is first detectable on the 18th day of gestation in rats. Prepubertal activities are similar for males and females. However, postpubertal sexual differentiation of enzyme activity occurs in which male activities are twice those of females. Postpubertal gonadectomy followed by hormone replenishment reveals that androgen and not estrogen is a direct modulator of the enzyme. Adult male rats castrated neonatally exhibit hepatic UDP-glucuronyltransferase activities intermediate between intact males and females. Hypophysectomy of adult male rats resulted in decreased microsomal UDP-glucuronyltransferase and abolished sex differences in enzyme activity; hypophysectomy had no effect on female UDP-glucuronyltransferase activity. A pituitary transplant under the kidney capsule followed by androgen treatment was not capable of reversing the enzyme effects of hypophysectomy, therefore suggesting that the male pituitary factor(s) responsible for positive modulation of UDP-glucuronyltransferase might

be under hypothalamic control in the form of a releasing factor. Neonatal administration of TP or diethylstilbestrol (DES) (1.45 μmoles on days 2, 4 and 6 postpartum) to intact control in the form of a releasing factor. Neonatal administration of TP or diethylstilbestrol (DES) (1.45 μmoles on days 2, 4 and 6 postpartum) to intact animals resulted in lowered UDP-glucuronyltransferase activity in liver microsomal fractions of adult male rats, whereas no changes were observed in the adult females and prepubertal male and female animals. Reproductive tract development and testicular function were also affected in those animals. Therefore, neonatal TP and DES administration apparently interfered with the normal sequence of postpubertal UDP-glucuronyltransferase sexual differentiation by altering normal imprinting of enzyme activity or by adverse effects on gonadal development.

Histidase. Hepatic histidase activity levels initially appear shortly after parturition, rise for several weeks, plateau during the postweaning period, then ascend again during puberty, more steeply in the female than the male; adult enzyme levels are attained which are approximately 3-fold higher in the female than in the male. Estrogen, glucocorticoids, glucagon and cAMP have been identified as positive modulators while thyroxine and testosterone (in the presence of estrogen) are negative modulators of histidase. Hypophysectomy of male rats resulted in increased activity levels but an ectopic pituitary is not capable of reversing the effect of hypophysectomy. For histidase, the pituitary is a negative modulator that appears to be dependent on a hypothalamic releasing factor. Neonatal DES or TP treatment resulted in decreased histidase activities in the adult female but no effect was seen in prepubertal male and female rats and in adult males. In contrast, similar treatment with TP had no effect on histidase. Dose response experiments demonstrate a 3-fold greater neonatal sensitivity to DES than to E_2. The action of neonatal estrogen treatment is demonstrated to be permanent and irreversible. Neonatal treatment with E_2, DES or TP resulted in decreased uterine wet weights in adult females (E_2<DES<TP<controls). Circulating sera estrogen levels were lower in adult E_2- and DES-treated females than in TP and control-females. Our results suggest that these alterations may be due to direct toxic effects on the postnatal development of the female reproductive tract and endocrine system and/or to organizational effects on nerve endings in the hypothalamus that result in programming for altered sexual differentiation of hepatic metabolism.

Monoamine oxidase. During ontogeny, rat liver monoamine oxidase activities gradually increase in both sexes until the pubertal period when female activities rise to almost 2-fold higher than male activities. Castration of adult male rats results in higher activity levels while testosterone administration reverses this role. Hypophysectomy results in elevated monoamine oxidase activities in both adult male and female rats. Transplantation of an ectopic pituitary into a hypophysectomized female reduces activities to those of intact female levels. Pituitary transplants into hypophysectomized males is without effect. Those findings suggest that a factor or factors secreted by the pituitary are responsible for the suppressing of monoamine oxidase activities. In the female, secretion is independent of the hypothalamus but in the male, secretion is dependent on hypothalamic control. MAO activity in the adult male is elevated by neonatal castration to a level similar to that of the adult intact female. Administration of TP or DES to neonatal castrates on day 2 prevented the rise in MAO activity observed in the adult male following neonatal

castration. E_2 or DHT were incapable of preventing this increase. These results support the theory of neonatal imprinting of hepatic metabolism. TP and DES possess two common characteristics which distinguish them from the hormones which fail to imprint adult MAO activity levels. First, neither TP nor DES bind significantly to α-fetoprotein and second, both TP (after aromatization) and DES can act as estrogens, DHT on the other hand is incapable of A-ring aromatization and E_2 is sequestered by α-fetoprotein.

Neonatal Exposure to DDT. Treatment of neonatal rats on days 2, 4, and 6 following parturition with 1 mg or 3 mg o,p'-DDT results in induction of hepatic glutathione S-transferases, and P-450 content but not of UDP-glucuronyltransferase and monoamine oxidase in 21 day old animals. Glutathione S-transferases, P-450 content and UDP-glucuronyltransferase were not effected in 63 day old animals but hepatic monoamine oxidase activity levels were elevated (feminization). These results demonstrate that o,p'-DDT administered neonatally causes an "activational" effect on P-450 content and glutathione S-transferases and suggest that o,p'-DDT administered at the critical period of development may exert a programming effect to result in altered ontogeny of hepatic monoamine oxidase. Experiments to confirm this hypothesis and to study the effect of methoxychlor (non-persistent DDT analog) under similar conditions are being conducted.

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.

Future Plans:

We are currently expanding these investigations to the measurement of circulating gonadotrophins, pituitary hormones and specific receptors and correlating behavioral changes between control and treated animals. We are initiating *in vitro* studies to extend our present ongoing *in vivo* mechanistic investigations. Using established hepatoma and pituitary cell lines, we are studying the effect of hormones, pituitary extracts and hormonally active xenobiotics on metabolism. This *in vitro* model will enable us to better elucidate the role of the pituitary in the regulation of hepatic metabolism and the mechanism whereby chemicals can alter this process. In addition to the use of sensitive hepatic enzyme markers for detecting altered sex differentiation we are investigating the use of serum enzyme (cholinesterase) and diagnostic chemistries (cholesterol and triglycerides) as non-invasive indicators for changes in hepatic function brought about by xenobiotic exposure.

Significance to Biomedical Research and the Program of the Institute: One of the dominant biochemical aspects of the orderly development of an organism from fertilization to maturity is the continuous progression of enzyme protein for-

mation ultimately resulting in specific enzymatic patterns which constitute and determine the metabolic machinery characteristic of each mature differentiated tissue. The postnatal development of sex-dependent hepatic mammalian enzymes is an excellent system to investigate ontogeny. It is the purpose of our studies to further our understanding of the factors responsible for complex postnatal developmental patterns. The fetus and newborn are exposed to numerous factors that can have deleterious effects on the developing offspring. The studies reported above are examples of alterations of enzyme expression that may be markers for abnormal differentiation and development. These effects on enzyme development may result in an altered capacity to metabolize potential teratogens, carcinogens, or drugs and, therefore, allow humans to be more susceptible to biochemical insult. An understanding of these biochemical mechanisms should allow us to make predictions of chemicals that might alter normal biochemical functions of the liver.

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2. Tilson, H. A. and Lamartiniere, C. A.: Neonatal exposure to diethylstilbestrol affects the sexual differentiation of male rats. Neurobehavioral Toxicol. 1: 123, 1979.

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4. Illsley, N. P. and Lamartiniere, C. A.: Prenatal programming of hepatic monoamine oxidase by 5,5-diphenyl hydantion. Biochem. Pharm. 28: 2585, 1979.

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6. Dieringer, C. S., Lamartiniere, C. A., Schiller, C. M. and Lucier, G. W.: Altered ontogeny of hepatic steroid-metabolizing enzymes by pure PCB cogeners. Biochem. Pharm. 28: 2511, 1979.

7. Illsley, N. P., Kita, E. and Lamartiniere, C. A.: The role of the pituitary in modulation of hepatic monoamine oxidase. Endocrinology 106: 798, 1980.

8. Illsley, N. P. and Lamartiniere, C. A.: The imprinting of adult hepatic monoamine oxidase levels and androgen responsiveness by neonatal androgen. Endocrinology (accepted).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70200 -06 LOFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
The Morphologic and Biochemical Effects of Toxic Metals

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

PI:	B. A. Fowler	Head, Renal and Intracellular Toxicology	LOFT
OTHER:	N.G. Carmichael	Visiting Fellow	LOFT
	K.S. Squibb	Postdoctoral Fellow	LOFT
	A. Oskarsson	Guest Worker	LOFT

COOPERATING UNITS (if any)
K.R. Mahaffey, Research Chemist, FD — G.E.R. Hook, Research Chemist LPFT/NIEHS
D.E. Gardner, EPA/RTP — C.T. Post, Visiting Fellow LPFT/NIEHS
J.B. Pritchard, Research Physiologist LP/NIEHS

LAB/BRANCH
Laboratory of Organ Function and Toxicology

SECTION
Renal and Intracellular

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

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 time periods, 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 following prolonged exposure 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 or other toxicants and areas of interest include the biochemical effects of cadmium, pentenoic acid, arsenic, indium and lead on mitochondrial, microsomal and lysosomal structure and function. These studies have shown the central role played by renal lysosomes in mediating the uptake and toxicity of circulating cadmium-thionein to renal proximal tubule cells. Arsenate-induced alteration of hepatic mitochondrial and microsomal protein synthesis/degradation was found to be associated with changes in urea cycle enzyme activities.

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, plasma emission spectroscopy, and proton induced X-ray emission analysis.

MAJOR FINDINGS AND PROPOSED COURSE:

The Pathophysiology of Cadmium-Thionein Nephrotoxicity

Previous studies have demonstrated the nephrotoxicity of circulating cadmium-thionein (CdMT), however, the mechanism of this effect is unknown. The present study was designed to correlate defined morphological changes with changes in biochemical and physiological assessments of renal function. In rats injected intraperitoneally with 0.6 mg Cd as CdMT per kg body weight, ultrastructural changes in proximal tubule cells after 24 hr were characterized by an extensive vacuolation of the cytoplasm and an increase in small dense lysosomal-type bodies. Only in cells in latter stages of degeneration were signs of damage to brush border and mitochondria evident. By 3 days, there was evidence of complete cell necrosis and desquamation. The earliest physiological changes occurred in tubular reabsorption processes. A marked polyuria and proteinuria, characterized by a 2-3 fold increase in urine volume and a 5-10 fold increase in urine protein, respectively, and a decreased Na^{+} reabsorption were evident by 24 hr after CdMT injection. Urine creatinine excretion and glomerular filtration rates were not altered throughout the 5 days following treatment. Specific lysosomal protease activities were significantly increased by Day 3. Decreased p-aminohippuric acid accumulation in renal slices and elevated kidney/body weight ratios and serum creatinine levels were not significant until Day 5. These data suggest that normal tubular reabsorption processes are the earliest functional alterations induced by CdMT. Cellular changes occurring in response to CdMT uptake by proximal tubule cells at earlier time periods (<24 hr) will be examined to assess the mechanisms of toxicity of the CdMT. Urine protein patterns from CdMT-treated rats will be analyzed to determine the type and source of the urinary proteins and will be compared to protein patterns obtained from $CdCl_2$ treated animals.

Alteration of Hepatic Urea Cycle Enzymes in the Rat Following Prolonged Oral Arsenate Exposure

Previous studies have shown that exposure of rats to arsenate (As^{+5}) at 40 PPM in drinking water for 6 weeks produces marked alteration of hepatic mitochondrial structure and function associated with a 150% increase in protein synthesis. The present replicate study was undertaken to evaluate the impact of this change in protein metabolism on the hepatic mitochondrial urea cycle enzymes carbamyl phosphate synthetase(CPS), ornithine carbamyl transferase(OCT) and the cytosolic urea cycle enzymes Arginosuccinic acid synthetase (ASAS),

Arginosuccinic acid lyase (ASAL) and arginase (AEG). The most significant observed effects were a marked decrease (40%) in the specific activity of ASAS and increase in ARG (30%). In vitro addition of As^{+5} (10^{-2} to 10^{-5}M) to the ASAS and ARG incubation mixtures produced decreases in the specific activities of both enzymes. The results suggest that the observed inhibition of ASAS in vivo may be due to a direct effect of As^{+5} on the enzyme but that the observed increase in the specific activity of ARG in vivo was not due to activation of the enzyme by As^{+5} but rather a change in the synthesis/degradation cycle of ARG which controls the hepatic activity of this enzyme.

Alteration of Hepatic Urea Cycle Enzyme Activities and Blood Ammonia Levels by Acute Administration of 4-Pentenoic Acid in the Rat

Acute administration of 4-pentenoic acid (PA) to rats has been previously reported to produce marked elevation of blood ammonia and a clinical state similar to that observed in patients with Reyes' Syndrome. Male rats were given a single intraperitoneal injection of PA (200 mg/kg) and killed 30 minutes later. Activities of hepatic carbamoyl phosphate synthetase (CPS), ornithine carbamoyl transferase (OCT), arginosuccinic acid synthetase (ASAS), arginosuccinic acid lyase (ASAL) and arginase (ARG) and blood ammonia were measured. Specific activities of the mitochondrial enzyme OCT was found to be decreased by 15% as were those of the extra-mitochondrial rate limiting enzyme ASAS and ARG. The activities of CPS and ASAL were unchanged. These findings were associated with a 260% increase in blood ammonia and in situ hepatic mitochondrial swelling observed by electron microscopy. The results indicate that acute administration of PA alters hepatic urea synthesis via a mechanism involving inhibition of both mitochondrial cytosolic enzymes with a resultant marked perturbation of in vivo ammonia metabolism.

Characterization of a Low Molecular Weight Cadmium Binding Protein from Lung

Exposure of rabbits to a $CdCl_2$ aqueous aerosol at a dose of 1550 $\mu g/m^3$ every other day for three days resulted in production of a metallothionein-like protein in lungs. The protein has an elution profile by Sephadex column chromatography and DEAE ion change chromatography identical with the type I form of cadmium-thionein from liver or kidney. It has a UV spectrum with an absorbance at 250 nm but not 280 nm indicating an absence of aromatic amino acids. Disc gel electrophoresis disclosed an Rf of 0.4 which is similar to the type I form of cadmium-thionein. The role of this protein in the absorption and transport of Cd across the lung to target organs such as the kidney is under present study.

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 of potential applicability to populations living near fossil fuel power plants.

PUBLICATIONS

Woods, J. S., Carver, G. T. and Fowler, B. A.: Altered regulation of hepatic heme metabolism by indium chloride. Toxicol. Appl. Pharmacol. 49: 455-461, July 1979.

Fowler, B. A. and Woods, J. S.: The effects of prolonged oral arsenate exposure on liver mitochondria of mice: Morphometric and biochemical studies. Toxicol. Appl. Pharmacol. 50: 177-187, September 1979.

Carmichael, N. G. and Fowler, B. A.: Effects of separate and combined chronic mercuric chloride and sodium selenate administration in rats: Histological, ultrastructural and X-ray microanalytical studies of liver and kidney. J. Env. Pathol. Toxicol. 3: 399-412 December 1979.

Carmichael, N. G., Squibb, K. S. and Fowler, B. A.: Metals in the molluscan kidney: A comparison of two closely related species (Argopecten) using X-ray microanalysis and atomic absorption spectroscopy. J. Fish. Res. Bd. Canada. 36: 1149-1155, September 1979.

Fowler, B. A., Woods, J. S. and Schiller, C. M.: Studies of hepatic mitochondrial structure and function: Morphometric and biochemical evaluation of in vivo perturbation by arsenate. Lab. Invest. 41: 313-320, October 1979.

Carmichael, N. G., Squibb, K. S., Engel, D. W. and Fowler, B. A.: Metals in the molluscan kidney: Uptake and subcellular distribution of ^{100}Cd, ^{54}Mn and ^{65}Zn by the clam Mercenaria mercenaria. Comp. Biochem. Physiol. 65A: 203-206, January, 1980.

Ghafghazi, T., Ridlington, J. W., and Fowler, B. A.: The acute and subacute effects of sodium arsenite administration on in vivo carbohydrate metabolism. Toxicol. Appl. Pharmacol. (In Press)

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Fowler, B. A. and Nordberg, G.: Silver. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. pp 579-586, December 1979.

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Fowler, B. A.: Ultrastructural morphometric/biochemical assessment of cellular toxicity. In H. P. Witschi (ed.): Proceedings of the Symposium on the "Scientific Basis of Toxicity Assessment" Elsevier Publishing Company. pp 211-218 (March 1980)

Jones, H. S. and Fowler, B. A., Biological interactions of cadmium with calcium. Ann. N.Y. Acad. Sci. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70210-02 LOFT TERMINATED

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of TCDD on the Disposition of Heme and Heme-Containing Proteins in Mammalian Tissues

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

PI: K. T. Kitchin Staff Fellow LET/LOFT NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

~~Laboratory of Environmental Toxicology/Laboratory of Organ Function & Toxicology~~

SECTION

Hepatic and Endocrine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

These studies are designed to investigate the enhanced rate of benzo(α)pyrene metabolism following interaction of cytochrome P-448 obtained from TCDD treated rats with hepatic and renal microsomes low in endogenous cytochrome P-450 and MFO enzyme activity. TCDD is known to produce teratogenesis and fetal lethality in mammals as well as porphyria in a variety of species. TCDD also increases the activation of various exogenous carcinogenic hydrocarbons and alters the metabolism of endogenous steroid hormones in mammalian tissues. The present studies demonstrate that aryl hydrocarbon hydroxylase activity was increased 12, 26, 31 and 53 fold when 1.0 nmole of partially purified cytochrome P-448 was incubated with fetal liver microsomes, microsomes from kidney cortex of female rats and cumene hydroperoxide pretreated hepatic microsomes from female and male rats, respectively. CHP pretreatment increased both the absolute magnitude of and the relative increase in enhanced AHH rate in adult rat hepatic microsomes incubated with cytochrome P-448, while CHP pretreatment decreased these parameters in fetal hepatic and renal microsomes. Incubation with 1 nmole of exogenous cytochrome P-448 increased the AHH activity of NADPH-treated, lipid peroxidized microsomes by 76 fold, a value 20% higher than control microsomal AHH activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 71000-01 LOFT

PERIOD COVERED

October 1, 1979 to September 30, 1980

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

PI:	W. Powell-Jones	Visiting Associate	LOFT/NIEHS
Other:	G. Lucier	Acting Lab Chief	LOFT/NIEHS
	E. Lui	Visiting Fellow	LOFT/NIEHS
	C. Lamartiniere	Senior Staff Fellow	LOFT/NIEHS
	O. McDaniel	Bio. Lab. Tech.	LOFT/NIEHS
	C. Thompson	Graduate Student	LOFT/NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Organ Function and Toxicology

SECTION

Hepatic and Endocrine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	1.2	2.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 zeranol, DES, PCBs, DDT and methoxychlor are assessed for estrogenic potency in liver.

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 plot
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 non-specific 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 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 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 and isolated hepatocytes). 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 and in isolated hepatocytes.

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. Endpoints could be liver histopathology or dysfunction, cardiovascular disease or hypertension.

MAJOR FINDINGS

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.

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, 3 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 castra-

ted one day after birth and sacrificed as adults exhibit a disappearance in peak III and marked reduction in the level of peak V. Hypophysectomy of adult rats results in the abolition of EBP sex differences. These studies show that qualitative and quantitative differences in levels of 4S binding. The maintenance of this sex difference is pituitary dependent and may be programmed at birth through hypothalamic-pituitary axis by neonatal androgen exposure.

Investigations on the estrogenic potency of zeranol in liver have demonstrated that this mycotoxin competes as well as estradiol for high affinity binding sites in liver as examined by competitive binding and sucrose gradient studies. Pronounced differences in dissociation rates (zeranol-receptor complexes) were evident for various zeranol analogs.

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 the 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. Attempts will be made to study the genetic control of important estrogen binding proteins by using inbred strains of mice. 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 estrogens.

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 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.

PUBLICATIONS

Powell-Jones, W., Thompson, C. L., Nayfeh, S. N. and Lucier, G. W.: Sex differences in estrogen binding by cytosolic and nuclear components of rat liver. J. Ster. Biochem. (In press).

Lucier, G. W. and McDaniel, O. S.: Developmental toxicology of the organohalogens, effects on enzyme development. Ann. N.Y. Acad. Sci. 320: 449-457, 1979.

NATIONAL MARINE FISHERIES SERVICE - Beaufort, North Carolina 28516
(Interagency Agreement Between NIEHS and NOAA)

TITLE: The Physiological Effects of Arsenic, Cadmium, and Copper on Marine Shellfish

CONTRACTOR'S PROJECT DIRECTOR: David W. Engel, Ph.D.

PROJECT OFFICER (NIEHS): B. A. Fowler, Ph.D., Research Biologist
Renal and Intracellular Function and Toxicology Group

DATE CONTRACT INITIATED: July 23, 1975

CURRENT ANNUAL LEVEL: $75,000

PROJECT DESCRIPTION

OBJECTIVES: This study was undertaken to determine the physiological, biochemical, and ultrastructural effects of arsenic, cadmium, and copper on marine shellfish in comparison with those in mammals. Evaluation of pharmacokinetic and toxicological differences, in between similar doses of Cd administered as mammalian cadmium-thionein, oyster cadmium-binding protein or Cd^{+2} in rodents is to be accomplished.

METHODS EMPLOYED: Flowing seawater systems with continuous trace element injection, atomic absorption spectroscopy, radiotracer analyses, histological and ultrastructural techniques, cellular respiration studies, and proton-induced X-ray emission analyses.

MAJOR FINDINGS AND PROPOSED COURSE: Physiological, Ultrastructural and Biochemical Findings. The two most significant findings are concerned with the isolation and characterization of an apparently inducible cadmium-specific metal binding protein from oysters exposed to cadmium in seawater. This protein which is chemically different from mammalian metallothionein seems responsible for the known bioaccumulation of cadmium by these organisms and appears to play a role in determining cadmium toxicity to these animals. Recently completed studies have shown that concommitant administration of Se in seawater to oysters administered Cd resulted in a partial decrease in the deleterious effects of Cd on gill respiration but no discernable alteration in Cd binding to the previously described low molecular weight cadmium-binding protein. A second area where progress has been made concerns the role of $CaPO_4$ concretions and metal-binding proteins in kidneys of edible shellfish. The renal concretions have proven useful both in terms of explaining intensive accumulation of ^{54}Mn from fallout in bivalves but also in providing good biological standards for X-ray microanalysis since in situ metal analyses may be checked against analytical values derived by atomic absorption spectroscopy on concretions isolated by centrifugation from the same animals. Further ultrastructural/biochemical studies on the mechanisms of copper and cadmium toxicity to shellfish are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will provide a basis for evaluating mechanisms of uptake and cellular responses to chronic low level trace metal exposure in tissues of relatively simple organisms in comparison to those observed in mammals.

Data generated from this effort should also prove useful in evaluating differences in trace metal pharmacokinetics and toxicity following ingestion of metal contaminated marine shellfish. The future course of the project will involve the specified objectives of this interagency agreement in relation to understanding the subcellular mechanisms of trace element uptake and toxicity in marine organisms in comparison to mammals. This arrangement also provides a mechanism for estimating potential human risk following ingestion of metal contaminated marine shellfish.

PUBLICATIONS

Engel, D. W. and Fowler, B. A.: Copper and Cadmium-INduced Changes in the Metabolism and Structure of Molluscan Gill Tissue. In: W. B. Vernberg, F. P. Thurberg, A. Calabrese and F. J. Vernberg (eds.). Marine Pollution: Functional Responses. Academic Press, New York pp 239-256, July 1979.

Engel, D. W., Sunda, W. and Fowler, B. A. Factors affecting trace metal uptake and toxicity to marine organisms I. Environmental parameters. In: Biological Monitoring of Marine Pollutants. F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg (eds). Academic Press, New York (In press).

Fowler, B. A., Carmichael, N. G., Squibb, K. S. and Engel, D. W. Factors affecting trace metal uptake and toxicity to marine organisms II. Cellular mechanisms In: Biological Monitoring of Marine Pollutants. F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg (eds). Academic Press, New York (In press).

LABORATORY OF PHARMACOKINETICS

LABORATORY OF PHARMACOKINETICS
Summary Statement

The Laboratory of Pharmacokinetics has the responsibility for planning and developing a focus at NIEHS for pharmacokinetic studies and collaborative efforts applying pharmacokinetic principles to enable an understanding of where and why chemicals accumulate in living organisms and how these uptake, storage, and elimination processes are regulated or can be altered. To carry out this mission, Laboratory scientists plan and conduct studies to develop pharmacokinetic models for specific classes of chemicals in various species, and to develop strategies for extrapolating chemical behavior and toxicity from one species to another and to man. They plan and conduct studies on use of pharmacokinetic data to design better treatments for toxicities due to persistent chemicals, to evaluate body burdens of environmental chemicals, and to assess effects of different rates of exposure to environmental chemicals on toxicological outcomes. Scientists evaluate the role of metabolism in chemical accumulations across various species. They establish and maintain liaison with various groups in and out of government using pharmacokinetic approaches in toxicological evaluations.

There were two workgroups in the Laboratory. Early in the year, the workgroup in chemical disposition transferred to the Environmental Biology Branch. The reporting of its activities will be included with those for that branch. This group has as a major goal the design and conduct of studies on the disposition and pharmacokinetics of selected chemicals and chemical classes under study in the National Toxicology Program.

The other workgroup is involved with pharmacokinetic studies as applied to extrapolation of toxicologic data for use in risk assessment. This group 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. 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 primary objective of this group is to characterize the most appropriate pharmacokinetic transformations of applied dose for use in the statistical models.

Pharmacokinetics in Extrapolation and Risk Assessment

For carcinogens such as polycyclic aromatic hydrocarbons (PAH), 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 endpoint 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.

A general scheme was developed for incorporating carcinogen-DNA adduct formation into these models using benzo(a)pyrene (BP) as a model carcinogen. Dose response curves for formation of BP-DNA adducts and tumorigenesis are being simultaneously determined in lung and forestomach of A/HeJ mice. The repair of BP-nucleoside adducts is also being studied. A specific adduct, (+)-BP-7β,8α-diol-9α,10α-epoxide-guanosine adduct (BPDE I adduct) was shown to be the predominant adduct formed in mouse lung, and unlike other adducts, the BPDE I was not repaired in lung.

Animal treatments with antioxidants and with inducers of the enzyme system, aryl hydrocarbon hydroxylase (AHH), have been shown to inhibit tumorigenesis induced by BP and other carcinogens. Our studies have shown that there were positive and quantitative correlations between inhibition of BP-induced pulmonary adenoma formation in mice by the antioxidant, butylated hydroxyanisole, and the AHH inducer, β-naphthofavone, and formation of the BPDE I adduct in lung. Thus, these studies suggest that antioxidants and AHH inducers protect against BP-induced lung tumors by inhibiting the formation of the carcinogenic adduct.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 35003-02 LPK

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Parameters in Carcinogenesis: Their Alteration by Anti-carcinogenic Agents: Antioxidants

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

PI:	Alan G. E. Wilson	Visiting Associate	LPK NIEHS
	Marshall W. Anderson	Mathematician	LPK NIEHS
Other:	Medhi Boroujerdi	Visiting Fellow	LPK NIEHS
	Ada C. Kung	Visiting Fellow	LPK NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.6	0.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Animal treatment with various antioxidants is known to inhibit tumorigenesis induced by benzo(a)pyrene (BP) and other carcinogens. We studied effects of butylated hydroxyanisole (BHA on in vivo binding of BP to DNA and protein. BHA treatment of mice given BP resulted in decreases in total DNA binding by 21 and 32%, respectively, in lung and liver. The extent of reduction in total DNA-binding did not appear to correlate with inhibition of tumor formation. Analysis of the effect of BHA treatment on the nature of BP-DNA adducts showed that the adducts were affected to different extents. Amounts of (+)-BP-7β,8α-diol-9α,10α-epoxide-DNA (BPDE-I) adduct formed were decreased 56% in lung, while amounts of adducts due to recycled BP phenols, quinones and BP 4,5-oxide were unaltered. Thus, specific reduction in BPDE-I adduct appears to correlate with a 53% inhibition of pulmonary adenoma formation. Antioxidants appear to inhibit tumor formation in lung by specifically inhibiting amount of BPDE-I adduct formed. In rats predominant BP-DNA adduct(s) formed in lung and liver resulted from interaction of a recycled-BP phenol with DNA. Only small amounts of BPDE-I adduct were formed. Treatment of rats with BHA increased total DNA binding and amounts of all DNA-adducts formed.

PROJECT DESCRIPTION

METHODS EMPLOYED: Animals were treated with BHA (0.75% of diet, 18 days) and then ^{3}H-BP was administered to Spraque-Dawley rats (1.0 μm/Kg, i.v.) and A/HeJ mice (7 mg/mouse, p.o.). Animals were killed at appropriate times, rats (1 hr), mice (48 hrs), after BP administration. DNA was isolated from lung and liver by the phenol extraction procedure. Samples of DNA were enzymatically digested to deoxyribonucleosides and the BP-deoxyribonucleoside adducts analyzed by HPLC.

MAJOR FINDINGS AND PROPOSED COURSE: The in vivo covalent binding of BP metabolites to lung DNA and protein was decreased by 20 and 25% and to liver DNA and protein was decreased by (25 and 45%) in BHA-treated mice. Under the experimental conditions used, BHA inhibits the number of pulmonary tumors formed by 53%. Thus the reduction total DNA or protein binding does not appear to correlate with the observed inhibition of tumor formation. Analysis of the nature of the BP-nucleoside adducts formed showed that the major adduct was the BPDE I adduct. This adduct accounted for 40% of the total DNA binding in the lung and 6% in the liver. Smaller amounts of DNA adducts due to recycled BP phenols, BP quinones and BPDE-II (the cis isomer of BPDE-I) were observed in the lung. In contrast, the BPDE I adduct was the only adduct observed in the liver. There was also radioactivity at the beginning of the HPLC gradient (early-eluting peaks). These peaks are still uncharacterized and accounted for 40% of total DNA associated radioactivity in the lung and 80% in the liver. In the lungs of BHA-treated mice, BPDE-I was the only adduct that was affected by the BHA pretreatment. This adduct was decreased approximately 55%. There was a similar degree of inhibition of pulmonary adenoma formation. In the liver, BHA treatment significantly reduced amounts of all the adducts. The observed difference between effects of BHA on the amounts of adducts formed in lung and liver may be related to induction of glutathione transferases and epoxide hydrases in the liver, but not lung, of BHA-treated mice. Such enzymes are known to affect the amount of reactive intermediate available for interaction with cellular macromolecules such as DNA. The mechanism by which BHA reduces the BPDE-I adduct is apparently different from that for arylhydrocarbon hydroxylase inducers (e.g. βNF and TCDD) since BHA did not change the amount or nature of pulmonary or hepatic cytochrome P450. These results are consistent with the view that the formation of the BPDE-I adduct may be an obligatory step in BP-induced pulmonary adenoma formation. BHA appears to exert its inhibitory action by selectively decreasing the amount of BPDE-I adduct formed. However, these studies also indicate that other BP-DNA adducts are formed in vivo.

The forestomach of female ICR/Ha mice has been shown to be very susceptible to BP-induced neoplasia. Preliminary studies have commenced investigating the formation of BP-DNA adducts in the mouse forestomach. The effect on BP-DNA adduct formation of specific inhibitors of BP-induced tumorigenesis in the mouse forestomach will also be studied.

We have also studies the effect of BHA treatment on BP metabolism and DNA binding in the rat. The amount of quinone metabolites in the bile were shown

to be reduced in BHA treated rats whereas phenol and 4,5-dihydrodiol metabolites were increased. Glucuronides, rather than sulfates, predominated as the predominate conjugated forms in both control and treated rats. Changes in the nature and amount of BP-DNA adducts in the lungs, but not livers, of BHA treated rats appeared to correlate with changes in biliary metabolite profile. The cytochrome P-450 inducer phenobarbital (BP) produced markedly different changes in metabolite and DNA-binding profiles in vivo than that observed with BHA. This conflicts with in vitro studies which suggest a similar effect of PB and BHA on BP metabolism and DNA binding.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE:
Polycyclic aromatic hydrocarbons are ubiquitous environmental carcinogens. The carcinogenic action of these compounds, and many other carcinogens, can be inhibited by dietary antioxidants. To understand the mechanism of action of these agents would permit delineation of the importance of such agents in the diet as well as design of more potent anticarcinogenic agents. The presence in the diet of non-toxic anticarcinogenic agents, of high potency, presents a potentially challenging means of reducing environmentally induced cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35005-01 LPK

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Parameters in Carcinogenesis: Their Alteration by Anti-carcinogenic Agents: Aryl Hydrocarbon Hydroxylase Inducers

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

PI:	Marshall W. Anderson	Mathematician	LPK NIEHS
	Alan G. E. Wilson	Visiting Associate	LPK NIEHS
Other:	Medhi Boroujerdi	Visiting Fellow	LPK NIEHS
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COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Treatment of animals with inducers of the enzyme system, aryl hydrocarbon hydroxylase (AHH), has been shown to inhibit tumorigenesis induced by benzo(a) pyrene (BP) and other carcinogens. Under the same experimental regime in which β-naphthoflavone (βNF) inhibits BP-induced pulmonary tumors, a good correlation was found between the decrease in the amount of (+)-BP-7β,8α-diol-9α,10α-epoxide-DNA adduct (BPDE-I adduct) and inhibition in pulmonary adenomas. Formation of the diol epoxide I adduct has been suggested to be a necessary event in tumor initiation. In mice pretreated with 2,3,7,8-tetrachloro-dibenzodioxin (TCDD), another AHH inducer, the BPDE-I adduct was also decreased by more than 90%. These results suggest that treatment of animals with AHH inducers protects against BP-induced carcinogenesis by inhibiting the formation of BPDE I-adduct. Treatment of rats with β-NF or TCDD also decreased the BP-nucleoside adduct in lung and liver by more than 90%.

PROJECT DESCRIPTION

METHODS EMPLOYED: Animals were treated with the AHH inducers β-NF and TCDD. ^{3}H-BP was administered to Sprague-Dawley rats, 1.0 μm/kg i.v., and A/HeJ mice, 6 mg/mouse orally, and animals were sacrificed after an appropriate time interval. DNA was isolated from lung and liver by a modification of the phenol extraction procedure. Samples of the DNA were enzymatically digested to individual nucleosides. An HPLC procedure was developed to analyze for BP-nucleoside adducts.

MAJOR FINDINGS AND PROPOSED COURSE: The in vivo covalent binding to liver and lung DNA and protein was determined 48 hrs after oral administration of a carcinogenic dose of ^{3}H-BP (6 mg/mouse) to A/HeJ mice. Significant levels of covalent binding of BP to lung and liver DNA and protein was observed. Analysis of the BP-deoxyribonucleoside adducts showed that the major adduct was the BPDE I adduct. This adduct accounted for 40% of the total DNA binding in the lung and 6% in the liver. Smaller amounts of DNA adducts due to recycled phenols, BP quinones and BPDE-II were also observed in the lung, whereas the BPDE I-adduct was the only one observed in liver. In addition to these BP-nucleoside adducts, radioactivity associated with DNA eluted at the beginning of the H_2O: methanol gradient (initial peaks). These uncharacterized initial peaks have been previously observed by others, especially when DNA binding of BP and other carcinogens have been examined in vivo. In our study with untreated mice, the initial peaks accounted for 40% of total DNA-associated radioactivity in lung and for 80% in liver.

The in vivo covalent binding of BP was determined in mice treated with βNF. Under the experimental regime used βNF inhibits by 95% pulmonary adenoma formation. The amount of BP covalently bound to lung and liver DNA and protein was decreased by βNF treatment. However, the extent of inhibition of binding was not correlated with the effect on tumor formation. In contrast, an excellent correlation was observed between the decrease in amount of the BPDE-I adduct (90%) formed in βNF treated animals and the inhibition of tumorigenesis. In βNF treated animals the BPDE-I adduct was not observed in liver DNA. Most of the DNA-associated radioactivity in these animals was in the initial peaks. Treatment of A/HeJ mice with TCDD, a potent AHH inducer, also decreased the formation of BP-nucleoside adducts in lung and liver. In TCDD-treated animals no BPDE I adduct was observed in liver and it was decreased by over 90% in lung. As with β-NF-treated animals, most of the DNA-associated radioactivity was in the initial peaks.

We also examined the effect of β-NF and TCDD treatment on the in vivo formation of BP-nucleoside adducts in the Sprague-Dawley rat. In untreated rats, the major BP-nucleoside adduct in lung and liver was chromatographically identical to that generated from BP-phenol(s). This adduct accounted for 42% of total DNA-associated radioactivity in lung and 7% in liver. In lung, small amounts of BP quinones, BP-4,5-oxide, and BPDE adducts were observed whereas in the liver only the BP phenol adducts were present. In TCDD or β-NF-treated rats, the BP phenol(s) adduct(s) was not detected in lung and was reduced by 50% in liver. As was the case with mice, most of the DNA

associated radioactivity in lung and liver of β-NF or TCDD-treated rats was in the initial peaks.

These data would suggest that the inhibition of BP-induced pulmonary adenoma formation in β-NF-treated mice is related to the inhibition of BPDE I adduct formation in lung. TCDD pretreatment should also inhibit BP-induced pulmonary adenoma formation. Others have shown that TCDD pretreatment does inhibit BP-induced skin tumors. The reason for the inhibition of BPDE I adduct by β-NF and TCDD in mice is at present unknown. These in vivo results contrast with in vitro results in that incubation of BP with DNA and microsomes from β-NF or TCDD-treated animals give increased levels of BPDE I-adduct as well as other BP-nucleoside adducts. In any case, treatment of animals with AHH inducers plays a protective role in BP-induced tumorigenesis. It remains to be established if AHH induction itself is the key event in the anticarcinogenic action of these inducers.

Several studies are underway to further explore the relationship between AHH induction and inhibition of tumor formation by BP and other carcinogens. The reduction of specific carcinogen-nucleoside adduct formation by AHH induction is being investigated in other animal model systems. The mouse forestomach is being developed as a model system for carcinogen-DNA binding studies since AHH inducers have been shown to inhibit tumors induced by a variety of carcinogens in this organ. The reduction of BPDE I adduct by AHH induction is being studied in other species, including monkey. We are also investigating the possibility that these AHH inducers also effect enzyme systems other than AHH which are involved in BP metabolism and subsequent adduct formation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITIUTE: Many chemicals which induce AHH inhibit carcinogenesis when administered prior to or simultaneously with the carcinogen. Understanding the mechanism of action of these anticarcinogenic agents would permit the rational design of more potent anticarcinogenic agents. In addition, these studies on the inhibition of carcinogenesis should provide some insight into the carcinogenic action of BP and similar environmental pollutants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35006-01 LPK

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacokinetic Parameters in Carcinogenesis: Quantification of Specific Carcinogen-DNA Adducts in Low-Dose and Species-to-Species Extrapolation of Carcinogenic Data

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

PI:	Marshall W. Anderson	Mathematician	LPK NIEHS
	Alan G. E. Wilson	Visiting Associate	LPK NIEHS
Other:	Medhi Boroujerdi	Visiting Fellow	LPK NIEHS
	Ada C. Kung	Visiting Fellow	LPK NIEHS

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

For carcinogens such as polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines, it is thought that specific DNA adducts are more important than total DNA binding in the initiation of carcinogenesis. Thus for these carcinogens, it is more appropriate that the amount of these specific carcinogen-DNA adducts be the endpoint of the pharmacokinetic study rather than the total amount of DNA binding. The amount of DNA adduct formed could therefore be viewed as the "effective" dose for the induction of cancer. The "effective dose" can then be incorporated into low dose risk estimation procedures. We developed a general scheme for incorporating carcinogen-DNA adducts into low dose risk estimation procedures. With benzo(a)pyrene (BP) as a model carcinogen, dose-response curves for tumorigenesis and BP-DNA adducts are being simultaneously investigated in lung and forestomach of A/HeJ mice. The repair of BP-nucleoside adducts is also being studied. For species-to-species extrapolation of carcinogenic data, it is likely that both the qualitative and quantitative nature of the carcinogen-DNA adducts are important. The formation of BP-nucleoside adducts is being investigated in several species.

PROJECT DESCRIPTION

METHODS EMPLOYED: Animals were treated with ^{3}H-BP and then sacrificed at various times. DNA was isolated from tissue by the phenolic extraction procedure and then enzymatically digested to individual nucleosides. An HPLC procedure was developed to analyze for BP-nucleoside adducts.

MAJOR FINDINGS AND PROPOSED COURSE: Our studies on the BP-induced pulmonary adenomas in A/HeJ mice are consistent with the concept that formation of the (+)-BP-7β,8α-diol-9α,10α-epoxide-guanosine adduct (BPDE I adduct) may be an obligatory step in BP-induced pulmonary adenoma formation. Inhibitors of BP-induced tumorigenesis, β-naphtoflavone and butylated hydroxyanisole, inhibits the formation of BPDE I adduct to the same extent as the formation of BP-induced pulmonary adenoma. No such correlation was obtained with total DNA binding, total protein binding, or with other adducts of DNA. Thus it is most appropriate that the amount of this specific BP-DNA adduct be the endpoint of the pharmacokinetic study rather than the total amount of DNA binding. A scheme for the incorporation of carcinogen-DNA adduct into the low dose risk estimation procedure was developed. In this scheme a relationship between applied dose, D, and the amount of carcinogen-DNA adduct, D', is required

$$D' = F(D)$$

where F denotes the pharmacokinetic transformation of applied dose D. A risk estimation scheme is then used with D' as the independent variable instead of D. Dose response curves for tumorigenesis and DNA-chemical adducts must be simultaneously obtained. The DNA-chemical adduct dose response curve must be extended to doses as low as the methodology permits. This type of investigation for the BP induced pulmonary adenoma in A/HeJ mice has been initiated. The tumorigenic data and some of the adduct studies will be obtained on contract. Several other carcinogenic model systems are also being considered.

We believe that both the qualitative and quantitative nature of the carcinogen-DNA adducts are important parameters in species-to-species extrapolation of carcinogenic data. We have shown that the BPDE I adduct was the predominant adduct in lung of A/HeJ mice after in vivo administration of a carcinoogenic dose of BP. Others have shown that this adduct is the major one observed in skin of mice after in vivo administration of a carcinogenic dose of BP. BP is thought to be carcinogenic in man and the BPDE I adduct has been observed in human organ explants of trachea and colon. Interestingly, unlike in the A/HeJ mouse, the BPDE I adduct was not the major adduct observed in the lung of the Sprague-Dawley rat. We know of no study which has shown that BP induces pulmonary tumors in this rat strain. Studies of BPDE I adduct formation in several other species are planned and a quantitative species-to-species comparison of this adduct in target and non-target tissues is planned.

Repair of BP-nucleoside adducts in lung and liver of A/HeJ mice after administration of a carcinogenic dose of BP has been investigated. The BPDE I adduct was rapidly repaired in liver. However, the BPDE I adduct was not repaired in lung, and thus, it appeared to be persistent in lung. The other

adducts studied were repaired in lung. We are at present examining the repair of BPDE I adduct at a much lower dose since pulmonary repair systems might have been saturated at the carcinogenic dose. We are also studying the repair of BP-nucleoside adducts in C57/B1J mice which are not as susceptible to BP-induced pulmonary adenomas. In any case, the repair of carcinogen-nucleoside adducts must be considered if the amounts of the adducts are to be used as a parameter in low dose and species-to-species extrapolations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In chemical carcinogenesis studies in animals significant numbers of tumors are typically found for many carcinogens only at concentrations orders of magnitude in excess of the human exposure level. Knowledge of dose-toxic relationships at low levels of exposure should therefore be the scientific basis for the regulation of environmental toxicants e.g. carcinogens. The importance of reliable dose-response relationships are self evident; they provide the basis for estimating the magnitude of risk at specified levels of toxicant exposure. For a toxicological evaluation, data obtained at high doses can be extrapolated to low dose levels by means of various statistical approximations. However, none of these 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 is therefore to characterize the most appropriate pharmacokinetic (PK) transformation of applied dose for use in the statistical models as well as in species-to-species extrapolation of carcinogenic data.

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. Toxicology and Applied Pharmacology (In press).

LABORATORY OF PHARMACOLOGY

The Laboratory of Pharmacology carries out research where pharmacological approaches and techniques are applied to studies of (1) processes of exposure to and disposition of environmental chemicals in comparison with drugs; (2) adverse effects of exposures of living systems to environmental chemicals as compared with drugs; (3) interactions between environmental chemicals and drugs and biological systems; and (4) the enzyme systems involved in metabolism of environmental chemicals and drugs. This research activity seeks to use the understandings of pharmacology to better elucidate the mechanisms by which environmental pollutants and effectors cause toxicity; to develop better test systems for detecting and evaluating early toxic effects of chemicals and environmental contaminants; to better understand extrapolation of chemical effects and toxicity in animals to man and the basis of species differences in chemical actions. The Laboratory is organized into 2 major divisions with several subgroupings--the Toxication-Detoxication Section and the Comparative Pharmacology and Physiology Section.

A. Toxication-Detoxication Section

This program has 2 subgroupings headed by Dr. Fouts and Dr. Bend, respectively. Dr. Bend's group is itself divided into at least 2 subgroups. Although such divisions are present primarily for administrative purposes, the overall activity of this section 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 the various--genesis processes--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. Considerable effort on our part is directed towards keeping fully informed about such activity and carefully choosing areas for research that we feel are most critical to understanding how these metabolic processes mediate environmental disease, and areas where other work is not being done or is being done differently. Our collaborative efforts demonstrate both our desire to share our expertise where we can, 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. Fouts' 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.

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 regio-selectivity 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, as well as localization of such systems in particular organs, cells, and parts of cells.

1. Progress in the last year

   a. Dr. Fouts' group

      (1) Xenobiotic metabolism in lung: Dr. Ken Jones and Ms. Theodora Devereux have worked on better methods for separating and isolating purer preparations of different kinds of lung cells from rats and rabbits, respectively. Both groups have concentrated on obtaining fractions rich in alveolar type II cells and Clara cells, especially since other studies have shown that these cells are relatively rich in xenobiotic-metabolizing systems. Thus, use of radioautography and fluorescent antibodies to P-450 have shown that systems activating 4-ipomeanol are especially rich in isolated Clara cells (in collaboration with Dr. Mike Boyd at NCI); and fluorescent antibody reactions show cytochrome $P\text{-}450_{I}$ and $P\text{-}450_{II}$ (as defined

by Dr. Philpot) to be present in type II cells and especially rich in Clara cells (work done in collaboration with Ms. Shull and Dr. Philpot). Both rat and rabbit lung cell populations are being studied since these species represent the extremes of pulmonary xenobiotic drug metabolism in several aspects. Thus, rabbit lung is especially rich in oxidative xenobiotic metabolic pathways and yet these systems are apparently non-inducible or poorly inducible with classical xenobiotic-metabolizing enzyme inducers. The rat pulmonary systems are almost undetectable in control animals, but respond to a variety of enzyme inducers. Both groups of investigations have made good progress in separating and isolating cell types and in characterizing these cells after isolation with respect to their morphology, pathways for xenobiotic metabolism, and viability. Some studies of isolated cells in culture as well as techniques for better yields of cells are described in the following reports. In addition, the use of a new microspectrophotofluorometer should allow quantification of xenobiotic metabolism in single cells of slices of lungs versus cells in suspension so as to assess damage during cell isolation and compare in vivo and in vitro behaviors, activities, etc.

(2) Xenobiotic metabolism in skin: Dr. Dieter Muller and Ms. Roberta Pohl have worked on a parallel series of projects (to those in lungs) using skin. Xenobiotic metabolism by enriched populations of cells isolated from mouse skin has been characterized in a number of ways. New methods for isolating cells from skin and obtaining fractions enriched in one type of cell have been devised. Studies in collaboration with Dr. Mike Boyd at NCI on slices of skin have shown that epidermal cells and cells surrounding hair follicles are especially rich in enzymes metabolizing 4-ipomeanol. It is hoped that use of the microspectrophotofluorometer will allow quantification of xenobiotic metabolism in single cells in skin in slices and in cells in suspension, in studies analogous to those in lung. These studies on xenobiotic metabolism in skin have already shown that skin is an organ whose chemical-metabolizing systems are quite different from those of other organs, are found in very few cells of the organ, and are responsive to a number of environmental constituents. The use of cells in culture and the study of purified or enriched populations of cell types may help answer the questions as to the importance of these systems in various skin diseases caused by environmental contaminants.

(3) Other projects: Other research in this group includes studies of xenobiotic metabolism during the perinatal period (with Dr. Philpot and Ms. Shull), thermolability of marine mixed-function oxidases (with Dr. Philpot and Ms. Shull), and xenobiotic metabolism and accumulation in marine species (e.g., by extrahepatic tissues in flounder--with Dr. Bend). These projects are relatively small in terms of manpower and scope, but lead to much collaborative effort and interdisciplinary approaches. These are parts of research efforts that at one time or another constituted major programs in Dr. Fouts' career (e.g., perinatal drug metabolism, began in 1954).

b. Dr. Bend's group

(1) Metabolism of epoxide and epoxide precursors: Isolated perfused lung and liver preparations continue to be used extensively in evaluating the metabolism and toxicity of epoxides and hydrocarbons. Studies completed this year by Dr. Brian Smith, Dr. John Plummer and Dr. Louise Ball have shown that there are several metabolic factors that are consistent with the selective pulmonary toxicity exhibited by polycyclic aromatic hydrocarbons (PAH). First, compared to liver, lung has only limited ability to conjugate oxidative metabolites of PAH with sulfuric or glucuronic acid or glutathione although it has cytochrome P-450-dependent monooxygenase activity. (Even so, the glutathione transferases were at least three times as active as epoxide hydrolase in the biotransformation of benzo(a)pyrene epoxides formed intracellularly in rabbit lung.) Second, lung has difficulty in eliminating oxidized metabolites of PAH, including the diols, some of which can be further metabolized to very mutagenic and carcinogenic species. Third, the lung can efficiently remove circulating arene oxides in a single pass making it a candidate for toxicity by relatively stable electrophilic metabolites formed in liver and released into the bloodstream. Similar studies, using HPLC separation and quantitation procedures developed in our laboratories, are now being used to study arene oxide and PAH metabolism in cells isolated from rabbit and rat lung (in collaboration with Dr. Jones, Ms. Devereux, and Dr. Fouts).

(2) Destruction of pulmonary cytochrome P-450: The administration of p-xylene to rabbits causes the destruction of approximately 50% of the pulmonary cytochrome P-450 but does not affect hepatic cytochrome P-450 content. The metabolic basis for the selective destruction of lung is being investigated by Dr. Plummer and Dr. Smith, in collaboration with Dr. Roland Wolf in Dr. Philpot's laboratory. A technique was developed which allows us to determine the ability of a chemical to destroy pulmonary cytochrome P-450 during perfusion through a lung which has one lobe ligated. Studies in this system demonstrated that all biochemical pathways necessary for formation of the toxic metabolite of p-xylene were present in lung. The metabolic profiles of p-xylene formed in perfused rabbit lungs and liver were compared in an attempt to learn something about the chemical nature of the toxic metabolite(s). No ring hydroxylated products were formed by the liver although some 2,5-dimethylphenol was formed by lung. This at least raises the possibility that a toxic arene oxide is formed by lung, which could destroy pulmonary cytochrome P-450. Further studies are in progress to test the effect of other alkylated benzene solvents on pulmonary cytochrome P-450, and to study the mechanism of this destruction with the most potent compound found.

(3) Regiospecificity and stereospecificity in the enzymatic reaction of glutathione and epoxides: For several years our laboratory has been systematically investigating the role of the glutathione transferases in the detoxication of electrophilic intermediary metabolites, such as the epoxides. Studies on the metabolism of benzo(a)pyrene 4,5-oxide in perfused liver and lung preparations over the past year demonstrated HPLC elution patterns that were consistent with the formation of isomeric glutathione conjugates in both biological systems. (This is not unexpected since trans addition of glutathione to this arene oxide results in two positional isomers, each with a pair of diastereoisomers.) In collaboration with Dr. Oscar Hernandez and Dr. Richard Cox of the Laboratory of Environmental Chemistry who synthesized the four possible conjugates, developed HPLC procedures to separate the mixture into 3 peaks (2 of them single diastereoisomers), and identified the conjugates by ^{13}C-NMR, we have demonstrated that rat liver cytosol exhibits high regioselectivity and stereospecificity in the reaction of (±)-benzo(a)pyrene 4,5-oxide with glutathione. These studies are currently being extended to various purified glutathione transferase enzymes from liver and lung, and to other alkene and arene oxide substrates.

(4) Since glutathione conjugates are formed from electrophilic chemicals in vivo, and this reaction is a major detoxication pathway, we have been investigating the subsequent metabolism and excretion of glutathione conjugates of epoxides. Prior to the initiation of such studies, the development of HPLC systems which would separate (and quantitate) the various intermediary thioether metabolites (glutathione, cysteinylglycine, cysteine and N-acetylcysteine derivatives) of compounds such as styrene 7,8-oxide was necessary. This was accomplished by Dr. Boris Yagen, in collaboration with Dr. Hernandez and Dr. Cox in the Laboratory of Environmental Chemistry. Dr. John Steele subsequently was able to investigate the metabolism of radiolabeled glutathione conjugates of styrene oxide in rats, and in isolated perfused liver, lung and kidney preparations from rats.

c. Dr. Philpot's group

(1) Cytochrome P-450 forms in rabbit lung: Two forms of cytochrome P-450 have been purified from rabbit lung, and these different proteins have been characterized using a wide variety of procedures. Dr. Shelley Slaughter has demonstrated that the peptides produced upon digestion with CNBr or several proteolytic enzymes are significantly different and that little, if any, structural similarity occurs. Analagous studies have demonstrated that cytochrome $P\text{-}450_I$ from rabbit lung is identical to the major hepatic form of cytochrome P-450 in the rabbit induced by phenobarbital administration.

(2) Localization of components of the monooxygenase system in rabbit lung: Ms. Cosette Serabjit-Singh, in collaboration with Dr. Charles Plopper, University of California (Davis), has utilized immunochemical techniques to elegantly demonstrate that the bronchiolar epithelial cells (Clara cells) of the lower airways contain cytochrome $P\text{-}450_I$, cytochrome $P\text{-}450_{II}$ and NADPH-cytochrome P-450 reductase. In fact, the intensity of the staining with all three antisera was much greater in Clara cells than in rabbit hepatocytes from either untreated or phenobarbital induced rabbits.

(3) Cytochrome b_5 requirement in microsomal metabolism of p-nitroanisole: Dr. Jane Croft and Dr. Roland Wolf have been comparing the hepatic and pulmonary metabolism of p-nitroanisole in the rabbit. This is an interesting substrate since oxidation can be efficiently catalyzed by NADH as well as by NADPH, the cofactor required for cytochrome P-450-dependent monooxygenase activity. In lung, greater than 50% of the NADPH-dependent metabolism

of p-nitroanisole is mediated by a cytochrome b_5-dependent pathway. The nature of this electron pathway from NADPH to cytochrome b_5 is currently being investigated in microsomal and purified monooxygenase systems of lung.

B. Comparative Pharmacology and Physiology Section

This section 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 section 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 mixed-function oxidase (MFO) system and other hydrolytic and conjugating pathways, including effects of pre-exposure to environmental contaminants on these enzyme systems. Where biologically significant induction is observed, the induced system is characterized in considerable detail. Because most marine species have lesser abilities than mammalian species to oxidize chemicals, the nonoxidative (e.g., conjugating) enzyme systems are being studied in several tissues of marine species.

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 an NIEHS center and a national focus for an aquatic pharmacology/toxicology program or emphasis--to promote awareness of and use of such aquatic species and systems in better understanding human disease and contributions of pollution to such disease.

1. Progress in the last year

a. Dr. Bend's group

(1) Monooxygenase system in spiny lobster: Dr. Margaret James and Dr. Peter Little have extended their studies on the monooxygenase system in the hepatopancreas of the spiny lobster, Panulirus argus. This is a biochemically interesting system since microsomes contain lots of cytochrome P-450 (about 1 nmol/mg protein) but do not oxidize xenobiotics very well. However, after the cytochrome has been partially purified and is reconstituted with mammalian NADPH-cytochrome P-450 reductase, it metabolizes xenobiotic substrates, including benzo(a)-pyrene, well. Metabolic pathways for environmental pollutants will be further characterized in this invertebrate species, which is an important human food.

(2) Study of enzyme induction as a sentinel system for certain chemical classes of pollutants in the aquatic environment: Dr. Bend and Mr. Gary Foureman are continuing to study, and attempting to determine causal relationships for, the heterogeneity of aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin activities in liver of winter flounder (Pseudopleuronectes americanus). They have demonstrated that more than 50% of the fish sampled have elevated AHH activities, and that the hepatic monooxygenase system in these is indistinguishable from that in flounder pretreated with PAH-type enzyme inducers. Thus, the possibility exists that the fish have been induced by exposure to an environmental contaminant. These studies will continue since they are closely involved with attempts to use biochemical responses (i.e., enzyme induction) in feral species as a sentinel system for various classes of pollutants in the aquatic environment. However, one of the current limitations of the use of such systems is that the enzyme systems studied have not been carefully and completely characterized in the sentinel species. By comprehensive examination of the hepatic monooxygenase system in winter flounder, we plan to overcome this shortcoming, and to determine if there is any merit in the use of flounder as economical monitors for pollutants that induce the synthesis of cytochrome P-448 in fish liver.

(3) Metabolism of carcinogenic PAH by aquatic species: Dr. James and Dr. Little have shown that hepatic microsomes from untreated sheepshead (Archosargus probatocephalus) form more benzo(a)pyrene 7,8-dihydrodiol from benzo(a)-pyrene than do mammalian species. This was also true

for a reconstituted system containing partially purified cytochrome P-450 from the spiny lobster. Results such as these indicate that detailed investigations are required to determine the further disposition and excretion of dihydrodiol metabolites of PAH in aquatic species used as human food sources. This is particularly true for compounds such as benzo(a)pyrene 7,8-dihydrodiol, which is only one metabolic step from the strongly carcinogenic and mutagenic 7,8-dihydrodiol-9,10-epoxide metabolites of benzo(a)pyrene. The risk of exposure to toxic pollutants from seafood taken from contaminated areas is real, and our studies are seeking to elucidate the biotransformation, storage and excretion mechanisms involved in aquatic vertebrate and invertebrate species.

(4) Regiospecificity and stereoselectivity in the enzymatic reaction of glutathione with (±)-benzo(a)pyrene 4,5-oxide: Mr. Gary Foureman purified one form of glutathione transferase from liver of the little skate, Raja erinacea, that is very efficient at catalyzing the conjugation of benzo(a)pyrene 4,5-oxide with glutathione. The purified enzyme was used for the biosynthesis of approximately 800 mg of ^{13}glutathione adducts from (±)-benzo(a) pyrene ^{13}C-4, ^{13}C-5-oxide using substrate enriched to various degrees at positions C4- and C5- of the arene oxide molecule. In collaboration with Dr. Oscar Hernandez and Dr. Richard Cox of the Laboratory of Environmental Chemistry, it was demonstrated that the skate liver enzyme exhibited high regioselectivity and stereospecificity. This project is being continued with arene oxides of various other PAH as substrates and with the four other glutathione transferases purified from little skate liver.

(5) Thermolability of fish monooxygenase activities: Dr. Shelley Slaughter, Ms. Bobbi Pohl, Ms. Cosette Shull, Dr. Philpot and Dr. Fouts have collaborated in a study to determine the basis for the greater thermolability of the little skate hepatic monooxygenase system as compared with that in mammalian species, such as the rabbit. The temperature sensitivity of MFO activities in skate liver microsomes correlated with decreased NADPH-cytochrome P-450 activities. Consequently, NADPH-cytochrome P-450 reductase was purified from liver of marine species (little skate and stingray) for comparison with the same enzyme activity purified from rabbit liver. The fish reductase (MW 74,000) was different from the rabbit reductase (MW 72,000) in response to moderate temperatures although both enzymes were equally sensitive to inactivation when exposed to elevated temperature under the same conditions. Currently, the importance of the lipid

components of the endoplasmic reticulum in modulating the inactivation of reductase by elevated temperature in skate hepatic microsomes, but not in rabbit hepatic microsomes, is being investigated.

b. Dr. Pritchard's group

(1) Transport mechanisms in aquatic species: Model systems are used to determine active transport, metabolism, plasma binding and intracellular binding and the role that each of these factors plays in determining the rate of renal xenobiotic excretion. Anionic herbicides were shown to be rapidly excreted by marine fish either unchanged or as the corresponding taurine conjugate. The high affinity of these compounds for the renal organic anion transport system of the proximal tubule, coupled with the less extensive plasma protein binding in fish relative to mammals, accounts for the rapid urinary excretion of these herbicides.

2,4-D and DDA were recently shown to inhibit uptake of organic cations into the choroid plexus, in spite of the fact that organic cations enter the plexus via a carrier-mediated transport system entirely distinct from the anionic system. It appears that the effects of 2,4-D and DDA on organic cation transport may be due to their ability to inhibit ADP-stimulated (State III) respiration, since a parallel dose-dependent inhibition of oxygen consumption in minced plexi and of organic cation transport occurred in the presence of either pesticide. The consequences of these anionic pollutants on choroid plexus function and on the central nervous system is currently being evaluated in collaboration with Dr. Lorcan O'Tauma, University of North Carolina School of Medicine.

In collaboration with Dr. David Miller of the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, isolated renal brush border membranes have been prepared from fish kidney and are being used to examine the interactions of xenobiotics with membrane permeability and transport. Detailed kinetic analyses of transport by these membrane preparations are performed to elucidate transport mechanisms, including the order of addition of substrate and cofactor, the affinity constants for substrate and cofactor binding, and the rate constants for elimination. Detailed characterization of various active transport systems (e.g., reabsorptive transport of glucose) are being performed prior to effects studies with model environmental pollutants. This approach is designed to give us considerable insight into basic

aspects of transport and the sites and mechanisms of toxicity.

(2) Altered membrane function as a basis of toxicity: Dr. Anthony Almeida is studying the effect of organochlorine compounds, such as DDT, on transport ATPases. He is trying to determine whether or not these chemicals exert their toxicity, which is extremely high in marine invertebrate species such as the blue crab, through inhibition of Na,K-ATPase and disruption of osmoregulation. Experiments performed over the last year demonstrated that ATPase inhibition by organochlorines appears to involve only one population (about 50% total activity) or that interaction occurs at a site distant from active enzyme sites (perhaps interaction with membrane lipid). Temperature studies, lipid substitution experiments and purification of Na,K-ATPase are underway to determine which component of the crab gill enzyme system is affected by DDT. If successful, this approach will be extended to other lipophilic pollutants including PCBs, aromatic constituents of crude oils and to heavy metals.

C. 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 such interdisciplinary research is carried out with a large number of scientists in residence at these laboratories. We also interact with groups at NIH, Bethesda--collaborative efforts of several groups in Pharmacology with Dr. Mike Boyd at NCI have been quite extensive this year. Dr. Pan (Department of Interior) and Dr. Fouts continue their collaborations. Continuing interactions with foreign scientists and labs have also occurred--e.g., of Dr. Fouts with Dr. Julian Leakey at the University of Dundee and with Dr. Zbinden at the University of Zurich, of Dr. Bend with Dr. Bengt Mannervik at the University of Stockholm, with Dr. John Caldwell at St. Mary's Hospital School of Medicine, University of London and with Dr. David Peakall of the Wildlife Toxicology Division of the Canadian Wildlife Service.

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.

Personnel

New additions to the Laboratory of Pharmacology during FY 80 were: Dr. Marguerite Coomes (Staff Fellow with Dr. Fouts), Dr. Dieter Muller (Visiting Scientist from Jena, East Germany, with Dr. Fouts), Dr. John Steele (Visiting Scientist from the University of Manitoba, with Dr. Bend), Dr. Larry Renfro (IGA Fellow from the University of Connecticut, with Dr. Pritchard), Dr. Liu Jianye (Guest Worker from People's Republic of China, with Dr. Bend). New technicians were Maria Rosario (with Dr. Fouts), John Holland (with Dr. Ken Jones), and Regina Angelo (with Dr. Pritchard). The new Visiting Fellow was Dr. Anja Norling (with Dr. Fouts).

Individuals leaving the Laboratory of Pharmacology included Dr. Shelley Slaughter, Dr. Roland Wolf, Dr. John Plummer, Dr. Dieter Muller, Dr. Louise Ball, Dr. Margaret James and Dr. John Steele.

Other Activities

Dr. J. R. Fouts: Adjunct Professor, Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill; Adjunct Professor, Department of Entomology, School of Life Sciences, N. C. State University, Raleigh. Member of the editorial boards of Molecular Pharmacology, Cancer Research, Xenobiotica, Pharmacology, Journal of Toxicology and Environmental Health, and Journal of Environmental Pathology and Toxicology. He is an Associate Editor of Pharmacological Reviews. He was chairman of one of the sessions at the Gordon Conference on Drug Metabolism and speaker at the Thursday evening session of that meeting in Plymouth, New Hampshire in July, 1980. Numerous lectures, speeches and seminars were delivered; among them--to the sophomore medical class at UNC; to the residents in pediatrics at Duke University; in the Triangle area course in toxicology; and to faculty and students at the University of Dundee, Scotland, and the University of Surrey, England.

Dr. J. R. Bend: Adjunct Associate Professor, Dept. of Entomology, North Carolina State University, Raleigh; member, Executive Committee of Faculty of Toxicology, North Carolina State University; member, Editorial Advisory Board for Drug Metabolism and Disposition; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee, 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 Ciba Foundation Symposium in Drug Metabolizing Enzymes and Environmental Chemicals: Toxic Interactions; at a Drug Metabolism Symposium sponsored by the Pennsylvania Drug Metabolism Discussion Group; at the 10th International Linderstrom-Lang Conference on Conjugation Reactions in Drug and Carcinogen Metabolism; at a Satellite Symposium of the Second International Congress of Toxicology on Freshly Isolated Cells from Adult Animals: Use in Biochemical Toxicology; at the Second International Symposium on Biological Reactive Intermediates; and at the International Conference on Veterinary Pharmacology,

Toxicology and Therapeutics. Gave seminars at ICI (Aldersley, UK), the University of Utah, University of Arizona, North Carolina State University and North Carolina Central University. Was NIEHS Coordinator for "Special Topics in Toxicology" a graduate course given for the second consecutive year in Research Triangle Park for students at Duke, North Carolina State and University of North Carolina).

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.

Dr. J. B. Pritchard: Trustee, Secretary and Member of Scientific Advisory and Nominating Committees, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Adjunct Associate Professor, Dept. of Pharmacology, University of Florida, Gainesville; invited speaker ASPET symposium on "Aquatic Animals as Models in Biomedical Research."

Dr. M. O. James: Adjunct Assistant Professor, Dept. of Pharmacology, University of Florida, Gainesville, Florida.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80001-08 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Microsomal Mixed-Function Oxidase Systems: Specificity and Function

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

PI:	Richard M. Philpot	Research Chemist	LP NIEHS
Other:	Cosette S. Shull	Chemist	LP NIEHS
	Charles R. Wolf	Visiting Associate	LP NIEHS
	Jane E. Croft	Visiting Fellow	LP NIEHS
	Shelley Slaughter	Staff Fellow	LP NIEHS
	Joseph Marciniszyn	Sr. Staff Fellow	LAG NIEHS

COOPERATING UNITS (if any)
Laboratory of Molecular Genetics; Laboratory of Environmental Biology; Dept. of Biochemistry, Scripps Clinic and Research Foundation; Dept. of Anatomy, School of Veterinary Medicine, University of California, Davis, CA

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3	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 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 objective of this work is 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 specificies 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) The rabbit lung contains cytochrome P-450

Two forms of cytochrome P-450 ($P\text{-}450_I$ and $P\text{-}450_{II}$) have been purified from rabbit pulmonary microsomal preparations. The substrate specificities, spectra, molecular weights, immunochemical and structural properties, and amino acid compositions of these two cytochrome P-450 isozymes are all significantly different. These enzymes have been partially digested with CNBr, papain, chymotrypsin and Staphylococcus aureus protease and the resulting peptides compared by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS). The patterns of the peptides produced are significantly different and little, if any, structural similarity is evident. This area of research will be continued by examination of the amino and carboxy terminal amino acid sequences of these proteins.

2) Rabbit pulmonary cytochrome $P\text{-}450_I$ and the hepatic form of the cytochrome induced by phenobarbital ($P\text{-}450_{PB}$) are the same enzyme

The substrate specificities, spectra, molecular weights, immunochemical properties, amino acid compositions and structural properties of cytochrome $P\text{-}450_I$ and $P\text{-}450_{PB}$ are the same. Peptides of identical molecular weight are produced from $P\text{-}450_I$ and $P\text{-}450_{PB}$ by digestion with CNBr, papain, chymotrypsin and Staphylococcus aureus. The amino terminal sequences of $P\text{-}450_I$ and $P\text{-}450_{PB}$ are the same (NH_2-MET-BLX-PHE-SER-LEU-) and the carboxy terminal acid is GLY in each case. The amino terminal sequence for $P\text{-}450_{II}$ is NH_2-MET-LEU-GLY-PHE-LEU-. Additional comparisons of the structures of $P\text{-}450_I$, $P\text{-}450_{PB}$ and $P450_{II}$ will be made.

3) The components of the rabbit pulmonary MFO system are highly concentrated in the non-ciliated bronchiolar cells of the lower airways

The distribution of cytochrome $P\text{-}450_I$, cytochrome $P\text{-}450_{II}$ and NADPH-cytochrome P-450 reductase in rabbit lung has been determined by the immunochemical techniques of immunofluorescence and immunoperoxidase. Antiserum to each highly purified enzyme was raised in goats. In the lung, the non-ciliated bronchiolar epithelial cells (Clara cells) of the lower airways show intense staining with all three antisera. Positive reactions with the antibodies occur in hepatocytes from untreated and phenobarbital-treated rabbits, but the intensity is considerably less than that observed in the Clara cell. The distribution of these enzymes in the lung is now being studied with respect to development, airway size and various in vivo treatments.

4) Cytochrome b_5 is an integral component of the electron transport pathway utilized in the metabolism of p-nitroanisole

The pulmonary and hepatic microsomal metabolism of p-nitroanisole (p-NA) can be supported by either NADH or NADPH. When NADH is used as the cofactor, all of the electron flow to cytochrome P-450 is via cytochrome b_5. Two molecules of cytochrome b_5 are oxidized and one molecule of NADH utilized for each molecule for p-nitrophenol produced from p-NA. When NADPH is used as the cofactor, the cytochrome b_5 pathway accounts for a significant (>50%) portion of the pulmonary, but not the hepatic, metabolism of p-NA. This major difference between the lung and liver is not a function of the ratios of the various enzymes. Present work in this area includes: (1) identification of the specific cytochrome component responsible for the metabolism of pNA; (2) elucidation of the nature of the electron pathway from NADPH to cytochrome b_5; and (3) purification of cytochrome b_5 and NADH-cytochrome b_5 reductase for use in reconstituted systems.

5) The binding of substrates to cytochrome P-450 involved substrate to enzyme ratios in excess of 1

Equilibrium dialysis experiments have shown that substrate to cytochrome P-450 ratios as high as 18 can be obtained. Under such conditions the magnitude of the spectral shift observed indicates that only about 30% of the enzyme has undergone a transition from low to high spin. This finding suggests that a high spin-low spin equilibrium exists when substrate is bound. The equilibrium between low and high spin is shifted towards the high spin by increases in temperature. The relationship between multiple binding and change in spin is now being examined.

6) The metabolism of carbon tetrachloride is not clearly associated with carbon tetrachloride-induced lipid peroxidation

Carbon tetrachloride-induced cofactor utilization and lipid peroxidation and carbon tetrachloride metabolism to chloroform have been examined in hepatic microsomal preparations from rat and rabbit. Results of these studies show that carbon tetrachloride-induced lipid peroxidation may not involve the metabolism of carbon tetrachloride. Studies in purified systems show that carbon tetrachloride-induced lipid peroxidation takes place without a requirement for cytochrome P-450. The nature of the free radical species responsible for initiating lipid peroxidation is now being investigated.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE" A full understanding of MFO systems is required if drug interaction, drug metabolism and disposition of ingested or inhaled environmental pollutants and pesticide detoxication are to be fully comprehended.

PUBLICATIONS

Patel, J. M., Wolf, C. R., and Philpot, R. M.: The interaction of 4-methylbenzaldehyde with rabbit pulmonary cytochrome P-450 in the intact animal, microsomes, and purified systems--destructive and protective reactions. Biochem. Pharmacol. 28: 2031-2036, 1979.

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Serabjit-Singh, C. J., Wolf, C. R., and Philpot, R. M.: The rabbit pulmonary monooxygenase system: Immunochemical and biochemical characterization of enzyme components. J. Biol. Chem. 254: 9901-9907, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80002-10 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems

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

PI:	Theodora R. Devereux	Research Biologist	LP NIEHS
	James R. Fouts	Chief	LP NIEHS
Other:	Reen Wu	IPA	LPFT NIEHS
	Richard M. Philpot	Research Chemist	LP NIEHS
	Cosette Shull	Chemist	LP NIEHS
	Brian R. Smith	Staff Fellow	LP NIEHS
	Louise M. Ball	Visiting Associate	LP NIEHS
	Ken Jones	Visiting Fellow	LP NIEHS
	Fred Talley	Head, Histology	LEB NIEHS

COOPERATING UNITS (if any)
Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

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)

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, and N,N-dimethylaniline N-oxidase. 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: Various enzyme preparations, including trypsin and pronase, have been 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 currently being 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 (< 2% Clara cells) and another fraction which is 30% Clara cells (about 10% type II cells). The alveolar type II cells have been further purified to 80% by metrizamide density gradient centrifugation. The Clara cells are now being separated further in either a two-polymer aqueous phase system (giving consistently 70% Clara cell purity but low yield) or a discontinuous Percoll gradient yielding more cells but variable purity. The alveolar type II cell fraction has been found to be enriched in 7-ethoxycoumarin deethylase activity but almost lacking in coumarin hydroxylase activity. However, both these activities were enriched in the Clara cell fractions. Efforts are now being made to prove whether or not different cytochromes exist in these separate lung cell populations. Immunological studies with cell fractions and antibodies to the purified cytochromes are being used to localize the cytochromes. Using immunohistochemical methods, we have demonstrated that both cytochromes P-450-I and P-450-II are present in type II and Clara cells. Xenobiotic metabolism is being measured in primary cultures of these cell fractions over time. Techniques to prevent mixed-function oxidase activity loss during culture are being studied. Also, attempts are being made to get pure cultures of alveolar type II cells and Clara cells started. Future research will focus on the study of toxication and detoxication mechanisms within isolated cell populations and in single isolated cells to understand the ways in which 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.

PUBLICATIONS

Devereux, T. R., Hook, G. E. R., and Fouts, J. R.: Foreign compound metabolism by isolated cells from rabbit lung. Drug Metab. Disp. 7: 70-75, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-07 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Chief	LP NIEHS
Other:	Dieter Muller	Visiting Scientist	LP NIEHS
	Michael Boyd	Section Head	CP, NCI
	Fred Talley	Head, Histology	LEB, NIEHS

COOPERATING UNITS (if any)
Biometry Branch; Histology Section

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication/Detoxication

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	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 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-glucuronosyotransferase 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 ultraviolet radiation, polycyclic hydrocarbons, chlorinated hydrocarbons, steroids, etc., are being investigated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Epidermal cells were isolated by tryptic digestion and separated into different populations by elutriator centrifugation combined with density gradient centrifugation. Homogenization prior to subcellular fractionation was accomplished by bombarding the cells with glass beads (Virtis glass bead impeller) in hypotonic suspension.

MAJOR FINDINGS AND PROPOSED COURSE: Several chemical and enzymatic procedures (dithiothreitol, EDTA, protease, thermolysin, α-chymotrypsin, collagenase, trypsin) for separating epidermis from dermis and releasing cells from the epidermis were evaluated for production of high cell yield while maintaining 7-ethoxycoumarin deethylase (7-EC) activity. Purified trypsin (Sigma, Type III) was selected (skins floated on 0.1% solution for 30 min at 37°C) for isolation of all epidermal cell types. Basal cells were selectively concentrated at the bottom of a Percoll gradient and identified by their small size, large nucleus to cytoplasm ratio and their distinctive staining for DNA and RNA content by methyl green and pyronin. The basal cells remained viable by dye exclusion tests through the isolation procedures and required three freeze/thaw cycles before dye uptake by all basal cells was observed. 7-EC activity by these cells was low, however, even when incubation mixtures containing freeze/thawed cells were supplemented with NADPH. A population of cells with low 7-EC activity was obtained in the first fraction from the elutriator. The remaining cells were a mixed population exhibiting high 7-EC activity. Further fractionation of these cells into more homogeneous populations will be undertaken. Methods for identifying spinous cells, granular cells and dendritic cells after their isolation will be developed. The xenobiotic-metabolizing enzyme systems of the active cell populations from the epidermis of control mice and from mice exposed to environmental agents will be characterized.

Autoradiographs of hairless mouse skin after incubation with tritiated 4-ipomeanol revealed radioactivity in the epidermis and in the sebaceous glands. Less activity was present in skin preincubated with piperonyl butoxide (an inhibitor of hepatic mixed-function oxidase). These experiments will be repeated in order to support these observations with quantitative data.

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

Pohl, R. J. and Fouts, J. R.: A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Anal. Biochem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80005-07 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Visiting Scientist	LP NIEHS
	Margaret O. James	Senior Staff Fellow	LP NIEHS
Other:	Louise M. Ball	Visiting Associate	LP NIEHS
	Gary L. Foureman	Biologist	LP NIEHS
	Roberta J. Pohl	Research Biologist	LP NIEHS
	Richard M. Philpot	Research Chemist	LP NIEHS
	Peter J. Little	Visiting Fellow	LP NIEHS
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	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 and Physiology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.8	1.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

We 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 microsomal mixed-function oxidases and alkene and arene oxide-metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control fish and in fish pre-exposed to environmental contaminants such as: polycyclic aromatic hydrocarbons, dioxins or polyhalogenated biphenyls. Some studies are being undertaken with purified cytochrome P-450.

PROJECT DESCRIPTION

OBJECTIVES: 1. To determine how different classes of chemicals, which act as inducers of hepatic xenobiotic metabolism in mammals, affect hepatic xenobiotic metabolism in marine species.

2. To further investigate xenobiotic-metabolizing pathways in the spiny lobster (Panulirus argus) and lobster (Homarus americanus), especially cytochrome P-450-dependent oxidation.

3. To measure taurine N-acyltransferase activity in several marine species and to characterize the enzyme in a suitable aquatic species.

4. To characterize the hepatic mixed-function oxidase (MFO) system and the glutathione transferases of representative species from Maine and Florida, before and after pretreatment with polycyclic hydrocarbon-type inducing agents.

5. 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, and to compare these effects in Maine vs. Florida.

6. To examine the MFO components and membrane dynamics of little skate (Raja erinacea) hepatic microsomes in order to explain the increased thermo-lability, during in vitro assay, of hepatic MFO 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. Further studies of the effect of 3,3',4,4',5,5'-hexachlorobiphenyl on sheepshead hepatic xenobiotic-metabolizing activities confirmed our earlier finding that this isomer was not as potent a polycyclic hydrocarbon-like inducing agent as it is in mammals. In sheepshead, 3,3',4,4',5,5'-hexachlorobiphenyl caused a doubling of cytochrome P-450 content and slight increases in MFO activity with benzo(a)pyrene (AHH), 7-ethoxycoumarin (7-EC), 7-ethoxyresorufin (ERF) and benzphetamine as substrates.

Our studies on polycyclic aromatic hydrocarbon induction of hepatic MFO activities in sheepshead (Archosargus probatocephalus) were continued by determining how water temperature and route of administration affected the time course of induction. After i.m. injection of 3-MC, 10 mg/kg, in summer

(water temperature 28°C), the rate of increase of cytochrome P-450 content and AHH, 7-EC and ERF activities was similar to those found after i.p. injection of 3-MC, 10 mg/kg. Both routes led to maximum induction 72 hours after the dose. However, the return to normal of cytochrome P-450 content, and AHH and 7-EC activities (but not ERF activities) was more rapid after i.m. than i.p. injection, suggesting more rapid elimination of 3-MC and/or turnover of protein after i.m. injection. We hope to further study the persistence of high ERF activity after cytochrome P-450 content and AHH and 7-EC activities have returned to normal, by isolating cytochromes P-450 at different times after 3-MC administration.

2. Cytochrome P-450 has been partially purified from spiny lobster hepatopancreas microsomes by chromatography of solubilized microsomes on DEAE-cellulose, phenylsepharose and hydroxylapatite. The preparations thus obtained contained about 10 nmole of cytochrome P-450 per mg protein. Contaminants were proteins with molecular weights of 25000-35000 daltons, and a small amount of epoxide hydrase. The cytochrome was reconstituted with pig liver cytochrome P-450 reductase, and used to study benzo(a)pyrene (BP) metabolism. HPLC analysis showed that the BP molecule had been oxidized at several positions, and that metabolites included the 7,8- and 9,10-dihydrodiols. We plan to further purify the lobster cytochrome and compare its physical and catalytic properties with those of mammalian cytochromes P-450.

The pig liver reductase used in these studies was purified according to published procedures. The same procedure was used to purify cytochrome P-450 reductase from sheepshead liver microsomes. The sheepshead reductase had a higher molecular weight than the pig reductase. We hope to investigate this further.

3. Preliminary studies showed that taurine N-acyltransferase activity from the mitochondrial matrix of stingray or red drum kidney would bind to a commercially available affinity column and could be eluted with taurine. We hope to use this method to obtain pure transferase enzymes from different species. We also hope to study the activation of carboxylic acids to their CoA derivatives--the first step in amino acid conjugation of non-nutritive carboxylic acids. Although spiny lobsters were shown to metabolize phenylacetic acid to phenylacetyltaurine *in vivo*, we found very low transferase activities *in vitro* . This may be for reasons similar to those which prevented measurement of MFO activity *in vitro* in hepatopancreas microsomes.

4. Hepatic microsomes from the sheepshead (*Archosargus probatocephalus*) and the southern flounder (*Paralichthyes lethostigma*) were found to produce an array of BP metabolites (dihydrodiols, diones and phenols) similar to that produced by rat liver microsomes; however, the untreated teleosts produced considerably more of the dihydrodiols which are normally associated with cellular toxicity (7,8- and 9,10-dihydrodiol) than the control rat. Pretreatment of the fish with the inducing agent 3-MC caused a tenfold increase in the rate of BP metabolism, but did not greatly alter the

pattern of metabolites formed. In the small number of southern flounder so far examined, the hepatic microsomal BP hydroxylase activity was low but was inhibited by α-naphtoflavone in untreated animals, suggesting that the animals had been exposed to an inducing agent while in their natural environment.

5. A constitutive terminal oxidase of the MFO system, cytochrome P-450, has been partially isolated with better yield and purity than previously from hepatic microsomes of the little skate, Raja erinacea, by the following optimized techniques: ion-exchange chromatography at room temperature on DEAE-cellulose, followed by hydrophobic interaction chromatography on octyl-sepharose, and finally gel filtration on Bio-Rad P-60.

The final product was almost free of contaminating yellow pigment, as judged by its absolute absorption spectrum, but still contained enough Emulgen 911 tightly bound to impede its reduction by sodium dithionite. Thus, quantitation by reduced-CO difference and protein estimations by Lowry's technique were unreliable. Molecular weights estimated by gel filtration were 53,000-60,000 daltons, and yields (based on absolute spectra) would be around 30% of the microsomal content. The cytochrome did not give type I spectrum with benzphetamine HCl; it did interact with aniline HCl and CCl_4, but so weakly as to suggest that the Emulgen already bound was interfering with the process also.

Alternate purification procedures are being tested to minimize detergent contamination of the cytochrome P-450, so that the catalytic properties of the hemoprotein can be studied in detail.

6. We again demonstrated that the hepatic monooxygenase system of more than 50% of the winter flounder (Pseudopleuronectes americanus) captured in Maine behaved as though induced by some polycyclic aromatic hydrocarbon-type chemical in the environment.

We propose to assay liver homogenates of these flounder with highly induced AHH and ERF activities for polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbon residue levels, which will be compared with pollutant content of livers from fish showing no induction. We will also use SDS-polyacrylamide gel electrophoresis to check livers of induced fish for a novel form or forms of cytochrome P-450, with catalytic properties similar to skate and rat cytochrome P-448.

7. NADPH-cytochrome c reductase (previously shown to exhibit temperature sensitivity which correlates with the temperature sensitivity of d-benzphetamine demethylase [BZPH] activity in hepatic microsomes from little skate or rabbit) was purified from little skate microsomes. It was different from rabbit purified reductase in molecular weight (skate, 74,000; rabbit, 72,000), in cross-reactivity with antibody to rabbit reductase and in behavior on a

5'-ADP affinity column. Purified rabbit and skate reductases were equally sensitive to inactivation of cytochrome c reduction by exposure to elevated temperatures, but when the reductases were reconstituted with rabbit hepatic cytochrome P-450 and synthetic phosphatidylcholine (PC) for assay of BZPH, greater temperature sensitivity of the system using skate reductase was observed.

For comparison with these results, NADPH-cytochrome c reductase was purified from hepatic microsomes of stingray (Dasyatis sabina), obtained from Florida Hepatic MFO from this species has been shown to exhibit a temperature "optimum" of 37°C during in vitro assay of biotransformation activity. The stingray reductase had a molecular weight of 74,000 and, when reconstituted with rabbit cytochrome P-450 and PC, exhibited temperature sensitivity for BZPH similar to that of the system reconstituted with skate reductase. Lipids extracted from stingray microsomes resembled those from rabbit microsomes in cholesterol content and percent saturation but resembled those from skate in elongated fatty acid content.

More detailed characterization of the reductase from little skate is planned. Further MFO reconstitution studies are also needed to elucidate the importance of the lipid components of membranes in modulating the inactivation of reductase by elevated temperature.

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 chain, 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 the animal, 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 environment contaminants.

PUBLICATIONS

James, M. O., Bowen, E. R., Dansette, P. M. and Bend, J. R.: Epoxide hydrase and glutathione S-transferase activities with selected alkene and arene oxides in several marine species. Chem-Biol. Interactions 25: 321-344, 1979.

Bend, J. R., Ball, L. M., Elmamlouk, T. H., James, M. O., and Philpot, R. M.: Microsomal mixed-function oxidation in untreated and polycyclic aromatic hydrocarbon-treated marine fish. In Khan, M. A. Q., Lech, J. J., and Menn, J. (Eds.): Pesticide and Xenobiotic Metabolism in Aquatic Organisms. ACS Symposium Series 99, 1979, pp. 297-318.

Bend, J. R., Foureman, G. L., Ben-Zvi, Z., Dostal, L., Koo, J. O., and Fouts, J. R.: Heterogeneity of hepatic benzo(a)pyrene hydroxylase (aryl hydrocarbon hydroxylase) and 7-ethoxyresorufin deethylase activities in individual winter flounder, Pseudopleuronectes americanus, from coastal Maine. Bull. Mt. Desert Island Biol. Lab. 18: 60-62, 1978.

Arinc, E., Pohl, R. J., Bend, J. R. and Philpot, R. M.: Biotransformation of benzo(a)pyrene by little skate hepatic microsomes: Stimulation and reconstitution of benzo(a)pyrene hydroxylase activity. In: Bilim Kongresi VI. Cevre Arastiomalari Grumu Tebligleri. Ankara, Tubitak. (Turkiye Bilimsel Ve Teknik Arastirnia Kurumu), 1979, pp. 309-316.

Bend, J. R., James, M. O., and Pritchard, J. B.: Aquatic toxicology. In Guthrie, F. E. and Perry, J. J. (Eds.): Environmental Toxicology. New York, Elsevier/North-Holland. In press.

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. In press.

Bend, J. R.: Induction of drug-metabolizing enzymes by polycyclic aromatic hydrocarbons: Mechanism and some implications in environmental health research. Presented at Ciba Foundation Symposium No. 76. Drug-Metabolizing Enzymes and Environmental Chemicals: Toxic Interactions. 1979.

Ball, L. M., Elmamlouk, T., and Bend, J. R.: Metabolism of benzo(a)pyrene in little skate mixed-function oxidase systems. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R. and O'Brien, P. J. (Eds.): Microsomes, Drug Oxidations and Chemical Carcinogenesis. New York, Raven Press, in press.

Lech, J. J. and Bend, J. R.: The relationship between metabolism of xenobiotic chemicals and toxicity in aquatic species. Symposium on Aquatic Toxicology. 18th Annual Meeting of the Society of Toxicology, New Orleans, March 1979. Environ. Hlth. Perspect. In press.

James, M. O. and Bend, J. R.: Effect of polynuclear aromatic hydrocarbons and polyhalogenated biphenyls on hepatic mixed-function oxidase activity in marine fish. Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment. Sponsored by the U. S. Environmental Protection Agency. Pensacola Beach, Florida, 1978. In press.

Bend, J. R., Stockstill, E. M., and Foureman, G. L.: Further characterization of hepatic aryl hydrocarbon hydroxylase induction in a native population of winter flounder, Pseudopleuronectes americanus, from coastal Maine. Bull. Mt. Desert Island Biol. Lab. In press.

Foureman, G. L. and Bend, J. R.: Purification of a glutathione S-transferase from little skate liver, preparation of an antibody to this purified enzyme and immunological similarity of the purified enzyme and hepatic and extra-hepatic glutathione S-transferase from several marine species from Maine. Bull. Mt. Desert Island Biol. Lab. In press.

Pohl, R. J., Serabjit-Singh, C., Fouts, J. R. and Philpot, R. M.: Thermolability of mixed-function oxidase activity in hepatic microsomes from little skate, Raja erinacea, and Rabbit. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R. and O'Brien, P. J. (Eds.): Microsomes, Drug Oxidations and Chemical Carcinogenesis. New York, Raven Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80006-09 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	John R. Bend	Visiting Scientist	LP NIEHS
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Other:	John B. Pritchard	Research Physiologist	LP NIEHS
	Gary L. Foureman	Biologist	LP NIEHS
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COOPERATING UNITS (if any)

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SECTION
Comparative Pharmacology and Physiology

INSTITUTE AND LOCATION
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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 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.

PROJECT DESCRIPTION

OBJECTIVES: 1. To study pathways for metabolism of carboxylic acids, e.g., 2,4-D, 2,4,5-T, DDA, phenylacetic acid, benzoic acid, 4-aminobenzoic acid, in marine fish and crustacea.

2. To study the metabolism of n-hexadecane in the spiny lobster.

3. To study the fate of ^{14}C-benzo(a)pyrene in the spiny lobster including the effect of seawater temperature on elimination.

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compounds, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored where appropriate. Metabolites are identified by standard procedures including high pressure liquid chromatography (HPLC). Spiny lobster (Panulirus argus) "urine" is obtained at the time of sacrifice by inserting a syringe into the bladder at the base of each green gland. Southern flounder (Paralichthyes lethostigma) urine is collected at timed intervals using an indwelling catheter.

MAJOR FINDINGS AND PROPOSED COURSE: 1. The polar urinary metabolite of ^{14}C-benzoic acid in southern flounder urine was isolated and shown to be benzoyltaurine. ^{14}C-p-Aminobenzoic acid was excreted more rapidly by flounder than was benzoic acid (roughly first-order kinetics, first half-life less than 24 hr) and was not extensively conjugated with taurine. Spiny lobster excreted part of the dose of 2,4-D, 2,4,5-T, DDA and phenylacetic acid rapidly in urine, and retained part of the dose in hepatopancreas as the taurine conjugate of each acid. These taurine conjugates were excreted slowly, through the digestive tract.

2. In studies in the summer (seawater 26° to 28°) the aliphatic hydrocarbon n-hexadecane (labelled 1-^{14}C) was found to be rapidly converted to more polar derivatives by the spiny lobster (Panulirus argus). Metabolism did not greatly increase the rate of elimination of the n-hexadecane as the metabolites were retained in the hepatopancreas (HP). Unmetabolized n-hexadecane was quantitated in the HP, tail muscle and hemolymph of animals sacrificed at various times. We plan to identify the n-hexadecane metabolites which are stored in the muscle and HP.

3. ^{14}C-Benzo(a)pyrene (administered by injection) was found to be relatively rapidly eliminated (63% per week) by the spiny lobster in the summer (seawater 26° to 28°) but was much more persistent in the winter (seawater 13° to 17°). Benzo(a)pyrene (BP) underwent rapid and extensive transformation to water-soluble derivatives in the summer study; we are presently identifying these BP metabolites. We shall also determine the structures associated with the BP-derived radioactivity in the tissues of the animals

of the winter study in order to determine the role of metabolism in vivo in regulating the rate of elimination of BP from the spiny lobster.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Marine pollution by chemicals such as crude and refined petroleum products, pesticides, polychlorinated biphenyls, polybrominated biphenyls, and polycyclic aromatic hydrocarbons is a problem of significant importance to human health since many aquatic organisms concentrate such pollutants and man relies upon these species for substantial quantities of food. Carefully controlled pharmacokinetic experiments will identify species that can be sampled in the field to monitor both short- and long-term pollutant levels. The study of interaction between environmental contaminants and other xenobiotics, including drugs, food additives, and pesticides, is important since mixtures of such pollutants are always encountered in the environment. Such investigations in marine species, with regard to competition for metabolic and excretion pathways or storage sites, may assist in understanding increased (or decreased) toxicity of pollutant-drug mixtures as they affect higher animals, including man.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80007-09 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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	Brian R. Smith	Senior Staff Fellow	LP NIEHS
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	James R. Fouts	Chief	LP NIEHS
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	Oscar Hernandez	Visiting Associate	LEC NIEHS
	Richard Cox	Chemist	LEC NIEHS

COOPERATING UNITS (if any)
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SECTION
Toxication/Detoxication Mechanisms

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.6	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)

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 mixed-function oxidases, 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 the two pathways for epoxide metabolism is being studied in vitro in intact pulmonary cells, and in isolated perfused rat liver and rabbit lung preparations.

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; 5) to investigate position-specific hydroxylation of model substrates in reconstituted mixed-function oxidase (MFO) and other in vitro systems; and 6) to investigate mercapturic acid biosynthesis in vivo in perfused organs and by pulmonary cells.

METHODS EMPLOYED: Differential centrifugation; spectroscopic, fluorometric, and radiochemical assays; organ perfusion procedures; ion exchange; thin-layer, paper, and high-pressure liquid chromatography; and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: 1) [Ring-^{14}C(U)]-Styrene oxide-glutathione conjugates (I) were synthesized and used to study metabolism and excretion in male Sprague-Dawley rats.

After an i.v. dose, a range of 5-15% of the dose of I was excreted in the bile of cannulated rats over 5 hr. From high-pressure liquid chromatography (HPLC) analysis, the major metabolites were shown to be the styrene oxide-mercapturic acids, but smaller amounts of styrene oxide-thioether conjugates of glutathione (I), cysteine and cysteinylglycine were also seen. Some minor unknown metabolites were detected in some, but not all, bile fractions.

Metabolites in urine and feces were determined similarly for a 48-hr period following tail vein injection of I. It was found that 85% (average of 4 rats) of the dose was excreted in urine with 0.7% in feces. The styrene oxide-mercapturic acids represented 88% of the total radioactivity excreted in urine, while other thioether conjugates and unknown metabolites represented the remainder of the urinary output.

Perfused isolated liver studies showed that 5-8% of the dose (I) was excreted in bile over 2.5 hr and identical values were found for phenobarbital (PB) pretreated rats. The major difference in the control and PB-induced rats was that the rate of formation of styrene oxide-cysteine conjugates was greatly accelerated in the PB-treated livers. Most of the cysteine conjugate was localized in the perfusion medium.

It is proposed that further studies be conducted with I in perfused isolated lung and isolated kidney systems to determine metabolic disposition and

routes and rates of excretion of these important thioether products in extrahepatic organs.

2) The techniques developed previously for the study of drug metabolism in the isolated perfused rabbit lung and for HPLC identification and quantitation of oxidative and water-soluble metabolites of polycyclic aromatic hydrocarbons and arene oxides were used to investigate the various routes of disposition in this organ following the administration of ^{14}C-benzo(a)pyrene (BP).

The major metabolite formed was 3-hydroxybenzo(a)pyrene (30-40% of total metabolites) but the lung appeared to possess only limited ability to conjugate this or the other hydroxylated metabolites present with sulphuric or glucuronic acid. Glutathione derivatives mainly S-4(5)-glutathionyl-5(4) hydroxy-4,5-dihydrobenzo(a)pyrene, were the predominant form of conjugated metabolites found (40%) and, compared to the quantities of dihydrodiol metabolites, indicated that the glutathione transferases were at least three times as active as epoxide hydrolase in disposing of arene oxides formed from BP in the isolated perfused lung.

After 1 hr the greater portion of the metabolites (60-90%) formed still remained concentrated within lung tissue rather than diffusing out into the perfusion medium. This suggests that lung may have difficulty in eliminating such metabolic products, which may contribute to its susceptibility to polycyclic aromatic hydrocarbon-induced carcinogenesis.

Similar procedures will be used to investigate the metabolism of BP in pulmonary cell populations enriched in Clara cells and alveolar type II cells.

3) In the rat, eight biliary metabolites of benzo(a)pyrene 4,5-oxide (BPO) were separated by the HPLC system previously developed. A metabolite previously thought to be a BPO-mercapturic acid was shown to be a mixture of isomeric BPO:cysteine conjugates by its identity to an authentic sample prepared by hydrolysis of BPO:glutathione conjugate.

In 60 min, rats excreted 48% of an intravenously administered dose of BPO in the bile. A mixture of isomeric BPO:glutathione conjugates was the major metabolite (62% of biliary radioactivity). BP 4,5-dihydrodiol glucuronide (26%) and BPO:cysteine conjugate (4%) were also quantitatively important biliary metabolites. The biliary metabolite profiles were the same at doses of 0.5 and 5 µmol of BPO, indicating that no metabolic or excretory pathways were saturated at these doses, and no cofactors (e.g., glutathione) were depleted.

4) After administration of 5 µmol BPO, a small proportion (3-4% in 12 hr) of the dose underwent enterohepatic circulation. BP 4,5-dihydrodiol glucuronide underwent enterohepatic circulation to a much greater extent than did the

thioether conjugates, which may have been further metabolized by the gut flora.

Biotransformation of BPO catalyzed by the glutathione transferases led to products which were readily excreted in the bile. On the other hand, BP 4,5-dihydrodiol, formed by hydration of BPO by epoxide hydrolase, was not excreted in the bile but accumulated in the liver until it was conjugated with glucuronic acid (a slow reaction), after which it was rapidly excreted.

5) A pharmacokinetic model was developed to describe arene oxide metabolism by intact pulmonary tissue. Clearance concepts initially developed to describe drug elimination by kidney and liver were utilized. The pulmonary extraction ratio for circulating BP 4,5-oxide was calculated to be nearly 1, which indicated that rabbit lung should remove a large percentage of circulating arene oxides in a single pass through this organ. The actual extraction ratio for BP 4,5-oxide was determined in single pass perfusion experiments to be 0.64 ± 0.4 ($\bar{x}$ ± SD, N = 3).

The six different cytosolic glutathione transferases of rabbit lung will be purified to homogeneity. The regiospecificity and stereospecificity of these purified enzymes will be determined using racemic benzo(a)pyrene 4,5-oxide and (+)- and (-)-styrene oxide as substrates. Antibodies to the rabbit pulmonary transferases will be produced by sensitizing goats (or turkeys) to the purified enzymes. The serum antibodies will then be used in conjunction with histoimmunofluorescence techniques to identify lung cell types which contain high glutathione transferase titers. The possibility of using histochemical procedures to quantify glutathione content in specific lung cell types will be investigated.

6) Glucuronide and sulfate conjugates of several BP and styrene metabolites were produced by biosynthetic methods to serve as chromatographic standards in several metabolic studies. These compounds include: styrene glycol β-glucuronide, BP 4,5-dihydrodiol β-glucuronide, BP 4,5-dihydrodiol sulfate, 3-hydroxy-BP-β-glucuronide, and 3-hydroxy-BP sulfate.

7) The metabolism of styrene glycol was investigated in isolated perfused rat livers to augment interpretation of data concerning styrene oxide metabolism in this system. The major styrene glycol metabolite was mandelic acid, while styrene glycol β-glucuronide was only a minor metabolite. Biliary excretion was a minor pathway for metabolite removal from perfused rat livers.

8) An isolated perfused rat kidney preparation has been developed to study biotransformation and excretion of water-soluble, thioether xenobiotic metabolites. Preliminary experiments with ^{14}C-styrene oxide:glutathione (I) conjugate demonstrate that rat kidney rapidly converts this conjugate to the corresponding cysteine derivatives.

Additional effort is required to guarantee the physiological integrity of the perfused kidney preparation prior to additional metabolic work.

9) p-Xylene metabolism was studied in isolated perfused rabbit lung and liver to clarify the mechanism of activation of this pulmonary toxin.

Isolated perfused rabbit liver cleared [^{3}H]-p-xylene (100 μmol) at an initial rate of approximately 0.1 μmol/min/g. The major metabolite was p-toluric acid. Only a small amount of p-toluric acid was released into the perfusion medium and this was soon reabsorbed by the liver and conjugated with glycine. Some tritiated water was formed, but no ring-hydroxylated products were observed.

Isolated perfused rabbit lung cleared p-xylene (100 μmol) at an initial rate of approximately 0.6 μmol/min/g, about half of this amount being exhaled. The major metabolite was p-methylbenzyl alcohol, some toluic acid and dimethylphenol also being formed.

These isolated organ studies do not support the proposal of earlier workers that p-tolualdehyde, formed in the liver and released into the circulation, is responsible for the pulmonary toxicity of p-xylene. Major differences in the metabolites formed in lung and liver do, however, suggest a metabolic basis for the organ-specific nature of p-xylene toxicity.

Metabolism of p-xylene will be investigated in reconstituted systems containing rabbit pulmonary cytochrome P-450 forms I or II to determine whether ring hydroxylation of xylene (observed in lung but not in liver) is catalyzed principally by the form not found in liver.

10) 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 inter-animal variations in pulmonary cytochrome P-450 levels. In this system, both p-xylene and p-tolualdehyde caused destruction of cytochrome P-450.

Overt damage (edema, blood clots) observed in the lungs by earlier workers after i.v. administration of p-tolualdehyde to rabbits was found to be an artifact resulting from the injection of a blood-immiscible liquid. Administration of p-tolualdehyde as a fine emulsion did not produce this effect, but still caused destruction of pulmonary cytochrome P-450.

The ability of other alkylated benzenes, in common use an industrial solvents, to destroy pulmonary cytochrome P-450 in the perfused rabbit lung is under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Xenobiotics or their chemically reactive metabolic intermediates (e.g., alkene or arene oxides) are detoxicated 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. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants and may also be related to chemical carcinogenesis and teratogenesis. 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 are of relevance to environmental health.

PUBLICATIONS

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Plummer, J. L., Smith, B. R., and Bend, J. R.: Biliary metabolites of benzo(a)pyrene 4,5-oxide in the rat. In Coon, M. J., Conney, A. H., Estabrook, R. W., Belboin, H. V., Gillette, J. R. and O'Brien, P. J. (eds.): Microsomes, Drug Oxidations, and Chemical Carcinogenesis. New York, Raven Press. In press.

Smith, B. R., Plummer, J. L., and Bend, J. R.: Pulmonary metabolism and excretion of arene oxides. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R. and O'Brien, P. J. (eds.): Microsomes, Drug Oxidations and Chemical Carcinogenesis. New York, Raven Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80031-04 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Role of Altered Membrane Function in Xenobiotic Toxicity

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

PI:	John B. Pritchard	Research Physiologist	LP NIEHS
Other:	Anthony F. Almeida	Visiting Fellow	LP NIEHS

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SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Many aquatic animals are highly sensitive to certain xenobiotic compounds. Such organisms are used as models to identify those physiological processes most sensitive to environmental pollutants. Particular emphasis is placed on membrane functions which underlie these processes. We have utilized as a model system the blue crab (Callinectes sapidus) which is extremely sensitive to organochlorine pesticides, particularly at low environmental salinity where its survival depends on its ability to osmoregulate. The objective is to establish the mechanism(s) responsible for the sensitivity of crustacea to organochlorine compounds. Parameters examined include 1) the levels of Na,K-ATPase in the gills after adaptation to various environmental salinities; 2) the ouabain binding characteristics of the enzyme; 3) the activity of Na,K-ATPase enzyme after administration of DDT both in vitro and in vivo; 4) the mode of inhibition of the enzyme in vitro; and 5) the ability of the intact animal to maintain hemolymph osmolality and ion levels after intravascular administration of DDT.

PHS-6040

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: Na,K-ATPase is routinely measured in freeze-dried, reconstituted homogenates of gill tissue by a modification of the method of Miller et al. (1975). Membranes enriched in Na,K-ATPase (microsomes) are also prepared by the method of Charnock et al. (1977) which employs Polytron disruption and subsequent differential centrifugation. The coupled, continuous optical assay of Schoner et al. (1967) is used to measure activity of the purified enzyme. Ouabain binding is determined by Millipore filtration. Hemolymph osmolality is measured by vapor pressure osmometry and ion content by flame photometry.

MAJOR FINDINGS AND PROPOSED COURSE: Biochemistry--Transport ATPases are very sensitive to organochlorine compounds in vitro. A variety of evidence suggests that the regulatory mechanisms dependent upon ion transport may be altered by these compounds. Using the blue crab which depends on Na,KATPase in the gill for its osmoregulatory ability, we are attempting to determine if lipid-soluble xenobiotics like DDT exert their toxicity (which is very great in crustacea) through inhibition of Na,K-ATPase and disruption of osmregulation. Aspects examined are the in vitro and in vivo sensitivity of the enzyme to organochlorines, the characteristics of Na,K-ATPase, and the relationship between Na,K-ATPase and osmoregulation.

We have previously shown that in most of its biochemical characteristics the blue crab gill Na,K-ATPase is similar to mammalian enzyme (bovine brain). However, it is less sensitive to vanadium and ouabain inhibition, apparently due to a lower affinity for those inhibitors and/or more rapid disassociation of the enzyme inhibitor complex. Using the partially purified enzyme and the continuous assay, we have been able to use small quantities of total membrane protein (<25 µg) to study the interactions of Na,K-ATPase with inhibitors. Under these conditions, we have confirmed the differential sensitivity of Na,K-ATPase from beef (I_{50} = 2.8 µM), blue crab (I_{50} = 28 µM) and white shrimp (I_{50} = 70 µM) to ouabain. At the same time, DDT inhibition was identical in the mammal and the crustacean. Significant (≥ 10%) inhibition was seen at 0.1 µM and half maximal inhibition was seen at ~1 µM for both species. Surprisingly, for such a potent inhibitor, maximal DDT inhibition in vitro in all three species is only 50%. Although DDT solubility is still very low even in the presence of 0.5% DMSO, we have been able to show that in this system DDT solubility and availability are not limiting at concentrations which yield maximal inhibition. Thus, this finding suggests that 1) ATPase inhibition may involve only one population of the enzyme (i.e., a DDT-sensitive ATPase) or 2) interaction may occur at a site distant from the active

site and lead to a change which only partially blocks or slows activity. One might envision that interaction with membrane lipids could lead to such a change. Clearly the effects of DDT are very different from the classical inhibitor ouabain, which acts at a specific site on the enzyme to block activity.

We will use temperature studies, lipid substitution experiments, and purification of Na,K-ATPase to determine which component of the enzyme is being altered. Along the same lines, we will attempt to use physical characterization of the membrane through differential scanning calorimetry to demonstrate more directly interaction of the pesticide with membrane lipids. If these studies prove fruitful, we expect to extend these studies to consider other lipid-soluble compounds including PCBs and specific components of oils such as naphthalenes or specific polycyclic hydrocarbons. Results may then be compared with the effects of agents like Hg which interact with specific functional groups on the enzyme.

Osmoregulation: Our previous work has shown that although Na,K-ATPase activity did correlate very well with the degree of osmotic stress, the blue crabs achieved their new steady-state serum osmolarity before gill Na,K-ATPase had changed significantly. At present, we hypothesize that existing ATPase may be activated by the stress to provide short-term compensation and that induction of new enzyme occurs in response to the new steady-state hemolymph osmolarity to provide the capacity to compensate for additional (i.e., new) osmotic stress. Clearly, we must define these processes further before we can determine how inhibition of Na,K-ATPase activities is translated into disruption of osmoregulation.

We will next attempt to correlate changes in ouabain binding with changes in Na,K-ATPase activity during osmotic stress to determine directly if greater activity is achieved before new sites are synthesized (measured by ouabain binding). We will attempt to determine if regulation of hemolymph osmolarity depends on a Na-coupled Cl transport rather than directly on Na^+ transport itself. Such a mechanism has been hypothesized for marine fish gill where transport is from blood to seawater. In our system where transport is from seawater to blood, the location of the Na,K-ATPase sites should be on the seawater side of the gill epithelial cell for such a system to work. Therefore, localization of the Na,K-ATPase through autoradiography with ^3H-ouabain would not only tell us a great deal about our own system, but could provide strong support for the concept of Na-coupled Cl transport hypothesized in several systems.

Once the system has been defined further we will be in a position to assess how the effects of DDT on a membrane event (Na^+ transport) impact upon the regulation of ion levels and to determine whether this system may be used to screen for the potential of a given compound to alter physiologically important membrane functions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Na,K-ATPase of crab gill is very sensitive to organochlorine pesticides, is anatomically located in an accessible part of the body, and plays an important role in the homeostasis of this animal. Thus, the blue crab provides an excellent model for the assessment of membrane toxicity of environmental chemicals.

The use of sensitive organisms or organ systems may permit identification of systems particulary prone to disruption by environmental contaminants. Such studies may then a) point to systems which might also be particularly prone to damage in man and b) serve as indicators or warning systems to the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants. The sensitivity of the Na,K-ATPase shown here may, in conjunction with the studies proposed above, not only indicate the mechanism of toxicity of organochlorine pesticides in an economically important human food, but it also suggests that the processes linked to Na,K-ATPase activity may be among the earliest and most important affected by DDT.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80032-04 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
Other:	Margaret O. James	Senior Staff Fellow	LP NIEHS
	Peter J. Little	Visiting Fellow	LP NIEHS
	Bruce Fowler	Research Pharmacologist	LOFT NIEHS

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Pharmacology

SECTION
Comparative Pharmacology and Physiology

INSTITUTE AND LOCATION
NIEHS/NIH/Research Triangle Park, NC 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)

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 and 2,4-dichlorophenoxyacetic acid (2,4-D). 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 transferases, in transport and toxicity of organic ions; and 4) the influence 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.

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: Two major considerations determine project design. First, we try to attack a given problem at several levels of organization from the intact animal to isolated tissues or membrane vesicle preparations in vitro. Thus, we intend to avoid the pitfall of placing unwarranted emphasis on an in vitro finding before its significance is evaluated in the intact animal. Second, we use the comparative approach. That is, we try to select a preparation, such as the isolated renal tubules of the flounder, which is particularly suited to the solution of a given problem. In practice this means that we attempt to define effects in simpler systems from lower vertebrates or invertebrates and then test the conclusions drawn by applying them to mammalian systems.

Two significant additions to our methodology should be noted. First, we have developed methods which allow us to prepare isolated renal brush border membranes (BBM) rapidly and reproducibly from teleost kidney. These methods are based on the Ca^{++} precipitation technique of Booth and Kenny (Biochem. J. 142: 574, 1974), but utilize much milder conditions. Our membranes, like those of Eveloff et al. (Am. J. Physiol. 237: F291, 1979) from winter flounder, require 2-3 mM Ca^{++} to retain transport capacity, a requirement not found for mammalian renal BBM. Second, using renal BBM and computer analysis, we have been able to perform detailed, quantitative kinetic analysis of renal glucose transport and to develop a model which accurately reflects binding and translocation of glucose and its cofactors by this carrier (see below). Initial studies have begun to characterize the interactions of xenobiotics with this system.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Metabolism and transport: Using both isolated renal tubules in vitro and clearance techniques in intact flounder, we have shown that the anionic herbicides (e.g., 2,4-D) are cleared very rapidly by marine fish as either the free acids or as their primary metabolites, the taurine conjugates, which are also anions at physiological pH. This rapid excretion is determined primarily by the high affinity of these agents for the renal organic anion transport system of the proximal tubule. Mammalian excretion of these compounds is much slower in vivo, despite significant transport capacity measured in vitro. Apparently, the

much more extensive plasma binding of these agents to mammalian plasma proteins, particularly serum albumin, limits their excretion relative to that achieved by the fish.

We will now use this same combination of techniques to investigate the influences of metabolism and transport on the elimination of more lipophilic agents. Initially we will focus on the representative polycyclic aromatic hydrocarbon, benzo(a)pyrene (BP), and its metabolites. Dr. Peter Little will collaborate on these studies. We will compare the renal handling of BP and its primary metabolites in both control flounder and those in which the P-450 mixed-function oxidase system has been induced. Using high pressure liquid 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. Since in these cold-blooded animals, 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 will be able to assess the relative impact of metabolism on both the excretion of the compound and the retention of toxic metabolites.

2. Choroid plexus: We have previously shown that in the choroid plexus, organic acid transport mediates the accumulation of the anionic xenobiotics, 2,4-D and DDA, just as it did in the kidney. Current work focused on the consequences of this accumulation in vitro and in vivo. Using the isolated choroid plexus in vitro, we demonstrated that these xenobiotics competitively inhibited transport of other organic anions, including transport of anionic neurotransmitter metabolites normally removed from the brain via the organic anion transport system. We have recently shown that 2,4-D and DDA also inhibit uptake of organic cations by the plexus. This was a surprising result since organic cations enter the plexus via a carrier-mediated system entirely distinct from the anion system and since well-transported cations did not alter uptake of the anionic xenobiotics. Therefore, we hypothesized that 2,4-D and DDA were capable of altering choroid plexus function by a second mechanism, probably disruption of an intracellular event subsequent to their transport and accumulation within the plexus. In view of their ability to uncouple oxidative phosphorylation in resting state mitochondria from kidney and liver, and to inhibit ADP-stimulated (State III) respiration, it seemed likely that the observed inhibition of cation transport reflected a similar effect on choroid plexus respiration. When oxygen consumption of minced plexi was measured, a dose-dependent inhibition was seen which paralleled the decrease in cation transport. Thus, a second mechanism capable of compromising choroid plexus function has been demonstrated.

The consequences of these effects on choroid plexus have begun to be evaluated in the intact animal using single injection and ventriculocisternal perfusion techniques. In collaboration with Dr. Lorcan O'Tauma (UNC, School of Med.), we have shown that transport of the neurotransmitter metabolite, 5-hydroxyindoleacetic acid (HIAA) is indeed inhibited in vivo. We have also shown that elevated HIAA levels alter the passive permeability properties of the ventricu-

lar walls and the underlying brain substance. While these studies are still preliminary they do suggest that active accumulation of foreign anions may have more far-reaching consequences in the central nervous system than previously suspected.

3. Isolated membrane vesicles: We have begun to use isolated renal brush border membranes to examine the interactions of xenobiotics with membrane permeability and transport. These techniques are very powerful since the isolated membranes retain the basic transport characteristics of the intact membrane, but are independent of cellular metabolism. We may then control electrical and concentration gradients across the membrane. Thus, we may look directly at interactions between the foreign compound and the carrier proteins. With the aid of a microprocessor we can perform detailed kinetic analyses of transport to show transport mechanisms, including the order of addition of cofactors and substrates, the affinity constants for substrate and cofactor binding and the rate constants for translocation. We may then look in a very detailed and mechanistic fashion at the interaction with toxic compounds. This type of information should give us a great deal more insight into the basic aspects of transport and the sites and means of toxicity. Dr. Darrell Wheeler of the Medical University of South Carolina has collaborated on the kinetic analysis and the development of the minicomputer software.

Our initial studies focus on the reabsorptive transport of glucose, a function of the luminal (i.e., brush border) membranes of the proximal tubule. We have shown that the best fit model may be depicted as follows:

	TRANSPORT							DIFFUSION
Binding	C + S	+	Na	CNa + S	+	Na	CNa_2 + S	K_D (S)
Translocation	Cs	+	CNa	CNaS	+	Na	CNa_2S	

At substrate concentrations of up to 10 mM and (Na^+) of 145 mM (i.e., the physiological range), 90-95% of the uptake was carrier-mediated; only 5-10% was by diffusion. At this Na^+ concentration, glucose transport occurred exclusively via Na-glucose co-transport. This conclusion is not new in itself, but our ability to quantify the various rate constants is a significant advance. We still have some additional work to refine the model, but we should very shortly be able to begin our analysis of the detailed effects of foreign chemicals on this transport system. We will concentrate on agents such as mercury, which have well-studied chemical reactivity. Thus, we expect to be able to correlate this reactivity with the specific effects on transport.

We have also recently begun to use isolated membrane vesicle preparations to study the mechanism of organic anion transport and the energy coupling of this process. These studies include the development of techniques which

have permitted us to incorporate the organic anion carrier into an artificial lipid membrane. With this capability we have an assay to be used in attempts to further purify the carrier protein(s) and study its interactions with substrates, inhibitors, and specific lipid or protein components of the membrane.

Finally, isolated membrane techniques have been applied to the study of the effects of cadmium on renal function. Here we have shown that while transport is depressed in the intact cell, no changes may be seen in the isolated membranes. Thus, membrane function itself has not been compromised, and the effects of cadmium must be on the ion gradients or energy supply which drives the transport. (Collaboration with Dr. B. Fowler)

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 their environment 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.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 80037-01 LP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Drug and Xenobiotic Metabolism in the Lung: Mechanisms and Modifying Factors

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

PI:	Kenneth G. Jones	Visiting Fellow	LP NIEHS
	James R. Fouts	Chief	LP NIEHS
Other:	Theodora R. Devereux	Res. Biologist	LP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

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 XX☒ (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 (e.g., as substrates) are those compounds, including the polycyclic aromatic hydrocarbons, which are activated by the mixed-function oxygenase system to their proximate and/or ultimate carcinogenic forms. The rat was chosen as the experimental animal because, like man, it is subject to induction of these metabolizing systems by prior exposure to 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 fractions and in individual cells may be carried out. Different lung cell types appear to have different intrinsic abilities to metabolize various chemical compounds, including benzo(a)pyrene and these activities may be inducible to different extents.

PROJECT DESCRIPTION

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. Both types of cells contain mixed-function oxygenase 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 to rats 48 hr prior to sacrifice causes induction of aryl hydrocarbon hydroxylase in whole lung homogenates and in both the Clara cell-enriched fraction and the alveolar type II cell fraction. It seems likely that, prior to induction, Clara cells can account for most of the aryl hydrocarbon hydroxylase activity in the lung, but after induction, type II cells may contribute a significant portion of the xenobiotic-metabolizing capacity of the lung. Efforts are being made to further purify Clara cells and to develop methodology for measurement of xenobiotic metabolism in individual cells using microspectrophotometric and microfluorometric techniques. Conventional radiometric and high pressure liquid chromatographic methods will be employed to determine whether purified populations of 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 they 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 mixtures of xenobiotics in the environment.

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

THE 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 pathology of the lung which will provide the scientific basis for designing rational measures for prevention of pulmonary diseases. To carry out its mission, the Laboratory of Pulmonary Function and Toxicology must encompass a diversity of scientific talents and interests including biochemistry, cell biology, immunology, toxicology, and pathology. Presently the LPFT consists of the following groups: endocrinology, prostaglandins, biochemical pathology, cell biology, 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, parental 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.

ENDOCRINOLOGY The research efforts in this program are focused on two major topics: 1) The biology and pathobiology of neuroendocrine cells located in the conducting airways and 2) peptide hormones contained in and possibly synthetized by mast cells.

The role of neuroendocrine cells in the lungs is unknown. Efforts in our, as well as other laboratories have so far failed to identify any endocrine

products associated with these cells. Neuroendocrine cells are thought to be the origin of oat cell carcinomas and of bronchial carcinoids in humans. Our studies have shown that the neuroendocrine cells contained in neuroepithelial bodies of hamster lungs increase in number, in response to repeated injections of diethyl nitrosamine. In contrast, the number of solitary neuroendocrine cells in the airways does not appear to change. Neuroendocrine-like cells were identified by ultrastructural and histochemical methods in short term cultures established from dissociated lungs of DEN-exposed hamsters. These cells showed ACTH-like immunoreactivity similar to human carcinoids when highly specific antibodies for ACTH were used in immunohistochemical localization studies.

Mast cells are commonly found in loose connective tissue including lung interstitium and bronchial submucosa. A variety of bioactive substances are known to be contained in mast cells. Our recent studies have shown that rat mast cells contain substances reactive with antibodies against ACTH, lipotropin and endorphin. Studies are presently underway to determine the biological activity of these substances contained in mast cells and to investigate their synthesis and release.

PROSTAGLANDIN RESEARCH. Prostaglandins and related substances including thromboxanes are derived from the unsaturated fatty acid, arachidonic acid (AA). During the past year one of the projects carried out in this program has been concerned with the metabolism of thromboxane-related fatty acids, controlled by thromboxane synthetase. Another related project has dealt with the control of intravascular platelet aggregation within the lungs, by the production of prostaglandins and thromboxanes by pulmonary endothelium.

Studies on the co-oxidation of xenobiotics by prostaglandin synthetase were continued. It was found that several aromatic amines including some insecticides such as aminocarb are oxidatively demethylated by prostaglandin synthetase. It was also found that the metabolic intermediate of Benzo(a)pyrene, namely BP-7,8-diol is oxidized during the conversion of AA to prostaglandins and that in human lungs the rate of AA-dependent formation of 7,10/8/9 tetral is at least as high as that of NADPH-dependent formation of the same tetral.

BIOCHEMCIAL PATHOLOGY. The research efforts in this program are concerned with the biochemistry of the acellular lining layer in the terminal airways in health and disease. It was shown that together with the surfactant and lamellar bodies which are secreted into the alveoli by the Type II cells, enzymes very similar to the enzyme complement of lysosomes are also secreted. Efforts have been made to pinpoint the localization of various enzymes within the lamellar bodies. In the course of these studies a new method was developed for the isolation of highly purified and morphologically intact lamellar bodies using a discontinuous sucrose gradient. This new method was utilized to obtain lamellar bodies to study the membrane fluidity of lamellar membranes.

In a separate study the lavage effluents from patients with pulmonary alveolar proteinosis were investigated. These effluents were found to contain large amounts of multi-lamellated myelin-like structures which are being analyzed in detail both ultrastructurally and biochemically. The basic

myelin-like structure was found to resemble the tubular myelin, found in the airways of normal human lungs. However, numerous abnormalities concerning organization and size were detected.

PULMONARY PATHOLOGY. Chrysotile asbestos is composed primarily of magnesium hydroxide (Mg) and Silicon dioxide (Si). Its toxicity has been linked to its Mg content because magnesium-leached fibers have shown reduced toxicity under a variety of circumstances. The initial studies in this program are aimed at establishing the proper procedures to measure Mg and Si in native asbestos fibers, and in fibers embedded in cells or tissues, using electron microprobe analysis. It was demonstrated that the Mg/Si ratio's can be reproducibly established in single fibers. The ratio was shown to change significantly in fibers exposed *in vitro* in 1N HCl and *in vivo* three months after fibers had been deposited in various tissue compartments in the lungs of rats. Studies on the deposition and translocation of inhaled asbestos are underway. Early results indicate that fibers deposited in the terminal airways probably reach the lung interstitium by way of intra- as well as inter-epithelial particle transport.

PULMONARY CELL BIOLOGY. The goal of this program is to study the life cycle and function of selected lung cell populations. Major emphasis is placed on developing the means to investigate epithelial cells from the conducting airways *in vitro*. Culture conditions are being established which allow us to study the mechanisms of epithelial cell differentiation and the regulation of specific cell products. Studies with rabbit tracheal epithelial cells have shown that removal of serum and its replacement with various hormones and growth factors result in establishment and maintenance of viable and proliferative cultures for at least a month. Rat tracheal cells were found to have several additional requirements including fibroblast conditioned medium and collagen-coated substratum. Immunochemical and ultrastructural evidence was provided for the epithelial nature of the cultured cells. Stimulation of glycoprotein synthesis of cultures grown in defined medium, by addition of retinoids was demonstrated. Studies are also carried out to assess the drug metabolizing activity of the cultures.

ENVIRONMENTAL CARCINOGENESIS. Several projects are underway in this program, most of which relate to the study of the nature of progression of neoplastic development. One series of studies is concerned with the hypothesis that neoplastic development is the result of genetic instability induced by the initial carcinogen exposure. These studies are carried out with normal and transformed human fibroblasts. Other studies are designed to investigate the transition of benign tumor cells from tracheal papillomas to malignant carcinoma cells and whether such transition occurs. Also, under investigation is the biochemical basis of invasiveness using biochemical assays for basement membrane degradation. The mechanism of promotion is studied by comparing cellular and biochemical events occuring in mouse skin and in hamster skin following TPA administration. The hamster epidermis is resistant to the promoting effects of TPA. Transformation and promotion studies are being carried out with rat tracheal cell cultures and rat tracheal cell lines. The main purpose of these studies is to determine the early stages of neoplastic development in epithelial cell transformation, using cellular, immunological and cytochemical markers. These studies are correlated with *in vivo* studies

using tracheal transplants as means to investigate neoplastic transformation, initiation and promotion of respiratory tract epithelium *in vivo*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25000-03 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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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Other:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)
Fred Talley, EBCB, NIEHS

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Laboratory of Pulmonary Function and Toxicology

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

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 occurring human cancers, is usually due to exogenous insult. The cells that are most likely to be progenitor cells for this carcinoma are the basal cells, which are rapidly dividing and/or differentiating, and as such make an excellent target cell for carcinogenic agents. The purpose of this work is (1) to develop an in vitro system consisting of dividing, basal cells isolated from hamster epidermis, (2) to induce these cells to undergo neoplastic transformation in vitro following treatment with environmental carcinogens and/or promoters, (3) to determine whether the two-stage model of carcinogenesis can be extended to Syrian hamsters are not sensitive to phorbol ester promotion of epidermal carcinogenesis.

PHS-6040

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. 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^{o}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-nercaptoethanol. 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: Studies to date have failed to demonstrate promotion of epidermal carcinogenesis of hamster skin by phorbol esters, which are potent promoters of mouse skin carcinogenesis. The basis for this lack of sensitivity by hamster epidermis to tumor promoters is under investigation. Hamster skin exposed to a single dose of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) responds in a manner analogous to mouse skin exposed to TPA. A transient hyperplastic response is observed with hamster skin increasing from one to two nucleated layers to as many as 5 or 6 layers 48 hours after treatment. Ultrastructural changes of hamster epidermis cells exposed to TPA are similar to those described in TPA treated mouse epidermis. By 96 hours after treatment with TPA, hamster skin is no longer hyperplastic but has a significant increase in the thickness of the stratum corneum. A significant difference exists between mouse skin and hamster skin exposed repeatedly to TPA for 4 weeks. Unlike mouse skin, there is no sustained hyperplasia of hamster skin after several weeks of TPA treatment. In fact, by 4 weeks the hyperplastic response in hamster epidermis is almost totally lost. The ability of hamster epidermis to adapt to TPA treatment and its failure to respond to multiple exposures of TPA is certainly critical to the insensitivity of this species to tumor promotion by this agent.

Hamster skin does not metabolize ^{3}H-TPA for 48 hours after a single treatment. Metabolism of ^{3}H-TPA by hamster skin after multiple treatments with TPA is under investigation. TPA receptors, prostaglandin production, and ODC activity of hamster skin will be studied after single and multiple exposures to TPA.

Epidermal cell cultures from newborn Syrian hamsters have been established and studies on neoplastic transformation of these cells in culture are in progress. These cells respond in vitro to TPA and other phorbol ester derivatives. TPA significantly stimulates cell division and inhibits cellular differentiation. Other phorbol ester derivatives have been studied and their inhibition of hamster epidermal cell differentiation correlates with their tumor promoting activity in mouse skin. Other nonphorbol ester tumor promoters are being studied with this short term assay.

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 cells would be of considerable value to the understanding of environmental carcinogenesis.

PUBLICATIONS

Barrett, J.C. and Sisskin, E.E.: Studies on Why 12-O-Tetradecanoylphorbol-13-acetate Does Not Promote Epidermal Carcinogenesis in Hamsters. In E. Pullman, P.O.P. Ts'o and H. Gelboin (Eds).: Carcinogenesis: Fundamental Mechanisms and Environmental Effects. D. Reidel Publishing Co., Dordrecht, Holland (1980), in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25001-03 LPFT

PERIOD COVERED
October 19, 1979 to September 30, 1980

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

PI:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
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	E. Elmore	Intermittent	LPFT	NIEHS
	T. Gray	Biologist	LPFT	NIEHS
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COOPERATING UNITS (if any)

Fred Talley, EBCB, NIEHS

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	3.75	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

The 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

PROJECT DESCRIPTION

METHODS EMPLOYED: $Fo1^+$ is a preneoplastic cell line derived from hamster embryo fibroblasts. 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.

Papillomas are induced in weanling hamsters (inbred strain of 15.16 or 87.20, Telaco) by injecting 0.5-5 mg of diethylnitrosamine either once or weekly for 5-10 weeks. After 8-12 months, a high percentage of the animals develop tracheal papillomas. The animals with such lesions are easily identified by their impaired breathing due to obstruction of the trachea by the neoplasms. The animals are sacrificed and their trachea removed. Tumors can be recognized macroscopically and prove to be exclusively papillomas by histological criteria. These tumors are excised with or without the cartilage rings of the tracheas and placed in culture with hams F-12 media supplemented with 10% fetal calf serum.

Basement membrane (BM) from Syrian hamster lungs is purified by an extraction procedure employing acetic acid and N-lauroyl 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 0.05M 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 0.1M Na Cl containing 1M urea. At each step of purification an SDS - polyacrylamide gel electrophoresis is done. An alternate procedure 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 1×10^6 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: The spontaneous transformation of Syrian hamster embryo cells in culture occurs by at least two steps. An intermediate state is represented by an aneuploid cell line (designated $Fo1^+$) which is preneoplastic. These cells differ from diploid cells by a number of properties, including their ability to become neoplastic when injected *in vivo* while attached to a substrate. These preneoplastic cells become neoplastic in culture, and growth in soft agar (Aga^+ phenotype) appears to signify neoplastic conversion and can be used as a quantitative assay for this transition. These

cells are currently being studied to elucidate the role of somatic mutation in the transition of preneoplastic cells to the neoplastic state.

To test the hypothesis that progressive neoplastic development is the result of genetic instability, which was induced by the initial carcinogenic event, mutation rates for normal diploid human cells KD and their transformed derivative (HuT-11) are being compared. Studies are in progress to determine the mutation rates, using a Luria-Delbruck fluctuation analysis, of the two cell types of codominant (Na^+/K^+ ATPASE), X-linked recessive (HPRT), and autosomal recessive (APRT) loci. Growth conditions have been optimized and the growth rates and cloning efficiencies of both cell types are about equal. The conditions for optimal selection for both Na^+/K^+ ATPase and HPRT mutants have been determined. Preliminary results indicate that HuT-11 cells have about a 2 fold or greater increase in mutation rate at the Na^+/K^+ ATPase locus. Chromosomal instability, another type of genetic instability, is being measured by determining the sister chromatid exchange frequencies of both cell types.

Tracheal papillomas were induced in Syrian hamsters by treating the animals with diethylnitrosamine (5 mg/treatment, 1-10 treatments). Cells from these tumors were then cultured in vitro and are currently being characterized. Using cloned papilloma cells in vitro, we hope (or possibly disprove) that cells from benign tumors are the progenitors of malignant cells. Furthermore, the availability of paired benign and malignant cells from the same clone will be a very powerful tool to determine the difference between malignant and nonmalignant cells. It is hoped that the elucidation of this difference will enable the development of quantitative assays to study the progression of benign cells to malignancy and the influence of environmental agents on this important biological event.

Invasive carcinoma seem to destroy basement membrane of the normal tissue. In our preliminary studies we have used a benzo(a)pyrene transformed fibroblast line BP6T for its ability to invade chorio-allantoic membrane of developing chich embryos. An examination of the ultrastructural effects (done by transmission electron microscopy) caused by invading tumor cells showed disruption and fragmentation of BM near the tumor cells. Therefore, BP6T cells were checked for their ability to degrade ^{3}H-BM in vitro. Interestingly, BP6T degreaded BM extensively and this degradation was linear for up to 24 hours during which time 90% of the BM had been solubilized. The normal fibroblast line (SHE) from which the BP6T line was derived did not show any degradation of ^{3}H-BM.

Since tumor cells are known to produce a number of proteases and collagenases, a study was done to find out if any hydrolytic enzymes are responsible for the degradation of BM. Earlier, the BP6T cells were shown to have fibrinolytic activity indicating the presence of high levels of plasminogen activator (PA) and hence the question of whether PA is involved in the BM degradation was probed. When the cells were grown in the presence of plasminogen-depleted serum, the hydrolytic activity was neglibible when compared to cells grown in

normal serum. When plasminogen was added back to the cell culture medium, this activity was regained showing that PA plays a role in the degradation of BM and thereby probably in malignant invasion. Studies are also being undertaken to see whether other chemically transformed cell lines, especially lines derived from carcinomas have the ability to degrade basement membrane. In addition, the role of other enzymes, such as a specific collagenase for type IV collagen, in this invasion process will be studied utilizing a number of carcinoma lines. For this, purified type IV collagen will be radioactively labelled and used as a substrate for invasion. An identification of a biochemical molecule such as a specific collagenase or a protease could then be used as a definitive marker for preneoplastic and benign and malignant cells.

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

Barrett, J.C.: Progressive Nature of Neoplastic Transformation of Syrian Hamster Embryo Cells in Culture. Prog. Exp. Tumor Biol. 24: 17-27, 1979.

Barrett, J.C., Crawford, B.D., and Ts'o, P.O.P.: The role of somatic mutation in a multistage model of carcinogenesis. In V.C. Dunkel and R.A. Mishra (Eds) Mammalian Cell Transformation by Chemical Carcinogens. Pathol. Publishing Co., (1980), in press.

Barrett, J.C., Sheela, S. O., and Kaunaga, K.: A reexamination of the role of plasminogen activator production for growth in semisold agar of neoplastic hamster cells. Cancer Research (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25002-03 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

PI:	V.E. Steele	Senior Staff Fellow	LPFT	NIEHS
Other:	P. Nettesheim	Chief	LPFT	NIEHS

COOPERATING UNITS (if any)
G. M. Cohen, University of Surrey, Surrey, England
A. C. Marchok, ORNL, Oak Ridge, Tennessee

LAB/BRANCH
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SECTION
Environmental Carcinogenesis

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	2.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Our major aim in these studies is to study the various phases of respiratory tract carcinogenesis at the molecular and cellular level using in vitro systems. We have previously developed an organ culture-cell culture system to demonstrate single and two-stage carcinogenesis in cultured rat tracheal epithelial cells. We found that nontumorigenic epithelial cell lines could be established from explants exposed only to the powerful tumor promoter TPA (12-O-tetradecanoylphorbol-13acetate). One of these cell lines was capable of metabolically activating benzo-(a)pyrene (B(a)P) and its 7,8 dihydrodiol form to become malignantly transformed. The proximal carcinogenic metabolite of B(a)P, diolepoxide I, was more efficient in transforming the epithelial cell line than the intermediate or parent compound. Inoculations of the transformed cell lines into immunosuppressed isogenic recipients produced differentiated carcinomas similar to those which occur in humans. These results indicate that tracheal epithelial cells can metabolically activate B(a)P, without the presence of other cell types, to become malignantly transformed. We are currently utilizing these epithelial systems to investigate mechanisms of chemical carcinogenesis and tumor promotion in airway epithelium.

FHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The nontumorigenic, pure epithelial cell line 2C1 was established following multiple exposures of an adult rat tracheal explant to TPA in vitro. At 24 hrs after plating, 10^5 attached cells were exposed for 24 hrs to the following polycyclic aromatic hydrocarbons: B(a)P, its proximal carcinogenic metabolite, the 7,8 dihydrodiol B(a)P, and the suggested ultimate form, diol-epoxide I B(a)P. The cultures were grown to confluency and subcultured weekly for 10 weeks. At this time 10^6 viable cells were inoculated into immunosuppressed rats. Tumors were removed at 2 cm diameter and histologically sectioned and evaluated.

MAJOR FINDINGS AND PROPOSED COURSE: After a 100 day observation period, only two of the eight sublines exposed to B(a)P formed tumors, while 5 of 8 sublines exposed to the 7,8 dihydrodiol B(a)P were tumorigenic. The suggested ultimate carcinogen, the diol-epoxide I form was 100% efficient in transforming 8 of the 8 exposed sublines, even at 1/100th the molar concentration of B(a)P. Control lines which were exposed to solvent only did not form tumors.

The cell cultures were monitored by phase contrast microscopy throughout passage of the sublines. No obvious morphological differences were observed between the control and carcinogen-exposed cells. Growth parameters such as colony forming efficiency and saturation density were also tested during subculture. These parameters generally increased during passage, but did not correlate with the ultimate tumorigenicity of the sublines.

Tumors from the 15 tumorigenic sublines have been examined and identified histologically as adenosquamous or squamous cell carcinomas. These differentiated epithelial tumors are similar to those found in human bronchogenic carcinoma. Tumors appeared at the inoculation sites but regressed when one subline of B(a)P exposed cells was inoculated. Later passages of this subline produced large invasive carcinomas. Microscopic examination of the inoculation sites of 3 of the 8 sublines exposed to B(a)P revealed small benign lesions or persistent nests of atypical epithelial cells. These sublines are currently being used to study a number of properties of premalignant or preneoplastic cells.

Further studies of B(a)P carcinogenesis have indicated that binding of carcinogen to the DNA of these cells can be measured. We are also investigating the effects of modulators of metabolism and binding on malignant transformation, and attempting to identify B(a)P-DNA adducts formed in the tracheal cell line.

Under an interagency agreement with Lawrence Livermore Laboratory (LLL) we are currently conducting studies to identify early cytochemical markers of carcinogen and cocarcinogen altered tracheal cells. Tracheal epithelial cells, either as primary cell cultures or nontransformed cell lines, are being partially or fully transformed by direct or indirect acting carcinogens at NIEHS. Dr.

M. Vanderlaan's laboratory at LLL has a well established expertise in flow-cytophotometric cell sorting and quantitative cytochemistry. Early "carcinogen altered" epithelial cell colonies will be examined at LLL for changes in cell cycle kinetics, ploidy, DNA content, cell size, and nuclear-to-cytoplasmic ratio (a good marker in vivo for dysplastic-preneoplastic lesions). These changes are being studied as a function of dose and time following exposure. Using data from studies of early cytochemical changes we will further study cells within populations having the greatest neoplastic potential. We will use flow sorting techniques to separate cells having common properties within large populations of exposed cells. After expanding these populations of cells in vitro we will assess their neoplastic potential by inoculating the cells into nude mice. These data will strengthen the predictive values of various lesions or markers following carcinogen or cocarcinogen exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Respiratory carcinogenesis is probably the most important example of carcinogenesis in man since its epithelium is the critical site for cellular interaction with a wide variety of airborne environmental contaminants. We are pursuing detailed in vitro mechanistic studies on the transformation of respiratory tract epithelium by environmental carcinogens. Also, we are studying early indicators of neoplastic progression for use as a diagnostic tool or a possible short-term carcinogen screening assay system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25004-03 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Establishing Pulmonary Epithelial Cells in Culture

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
Other:	L. Y. Chang	Biologist	LPFT	NIEHS
	D. Smith	Biological Aid	LPFT	NIEHS
	T. Devereux	Research Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Cell Biology

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

This project was initiated to investigate the life cycle and basic function of pulmonary epithelial cells in a well-defined in vitro situation. 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. We have achieved conditions for long-term culture of normal tracheal epithelial cells from rabbit and rat by supplementing culture medium with hormones instead of serum. These hormones and factors are insulin, epidermal growth factor, transferrin, and an extract fraction from bovine hypothalamus. We have shown further in rat tracheal cultures that cell growth and survival are dependent on fibroblast conditioned medium and collagen-coated substratum. Ultrastructural and histochemical analyses suggest the epithelial nature of cells grown in hormone supplemented defined condition. The addition of retinoids to rabbit tracheal cultures increases periodic acid-schiff stain positive cells and the synthesis and secretion of ^{3}H-glucosamine labeled glycoprotein. Similiar study is now carried out in isolated rabbit Clara cells. We have found that Clara cells are better maintained in culture in hormone supplemented medium than in serum condition.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHOD EMPLOYED: Cells were isolated from tracheal epithelium after pronase treatment. Clara cells were isolated from perfused rabbit lung after pronase digestion and by centrifugal elutriation. Fluorescin-labeled anti-keratin antibody stain was used on coverslip cultures at 37°C. Periodic acid-schiff stain of cultures was performed after the diastase treatment (22°C, 1 hour). Activities of various dehydrogenases were assayed according to changes of NADH or NADPH in reaction mixtures. Drug metabolizing activity was determined by the activities of coumarin hydroxylase and 7-ethoxyl coumarin deethylase.

MAJOR FINDINGS AND PROPOSED COURSE: Studies have shown that a drastic improvement in the maintenance of primary cultures of rabbit tracheal epithelial cells can be achieved by supplementing medium with hormones instead of serum. Continuous efforts in this respect have shown requirements for endothelial cell growth supplement derived from bovine hypothalamus, as well as for selenium in culture medium to maintain cell growth and survival.

Rabbit tracheal epithelial cells can be maintained for more than a month in the F12 culture medium containing epidermal growth factor, insulin, transferrin, selenium, and endothelial cell growth supplement. However, for rat tracheal epithelial cultures, we have observed additional requirements which include hydrocortisone, fibroblast conditioned medium, and collagen-coated substratum.

Morphologies of cells in culture are small and condensed. More than 90% of cells in culture are stained positively by fluorescein-labeled anti-keratin antibody, which strongly suggest the epithelial nature of cells in these cultures. Transmission electron microscopic studies show the formation of desmosomes, membrane interdigitation, and tonofibrils in cultured cells. No mucus granules or cilia can be found after a week in culture.

However, the addition of retinoids to rabbit tracheal cultures show an increase of periodic acid-schiff stained cells in culture and higher rates of glycoprotein synthesis and secretion. These results suggest that cells in hormone-supplemented medium still maintain some differentiated function.

Growth of tracheal epithelial cells in primary culture is rapid with generation times of 20 to 22 hours. Autoradiographic studies have shown that a high proportion of the cells in culture synthesize DNA.

Biochemical studies have shown rapid decrease in drug metabolizing enzymes and of glucose-6-phosphate dehydrogenase activities during the first 1 to 3 days in culture. However, a two-fold increase of lactate dehydrogenase and malate dehydrogenase in culture was found between 2 and 3 days in vitro. Variations were also observed in other enzyme systems.

Similar experiments were carried out with Clara cells in culture. We have found that cultures established from enriched Clara cell fractions require epidermal growth factor, insulin and transferrin for growth. Excessive serum

in the medium shows inhibitory effects of cells in culture. Studies to elucidate other hormonal requirements for Clara cell cultures are underway.

The precise origins and basic functions of these epithelial cells in culture remain to be established. It is also necessary to understand the fate of various cell types in culture. An important unsolved problem is to gain better control over the differentiated functions of these cells in vitro.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A large variety of foreign chemicals enter the body via the respiratory tract causing irritation, injury loss or change of function in the affected cells. Detailed study of the regulation of normal functions of respiratory tract cells as well as their disturbance by environmental toxicants in vivo is hampered by many problems. It is therefore highly desirable to develop in vitro systems which offer the possibilities for analysis of normal and abnormal cellular reactions under controlled conditions. This will allow us to probe into specific questions related to the biochemical and cellular basis of various pulmonary diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25005-03 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Synthesis and Release of Polypeptide Hormones by Mast Cells

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

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	R. I. Linnoila	Visiting Associate	LPFT	NIEHS
	R. Wu	Research Chemist	LPFT	NIEHS
	G. Jahnke	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

M.D. Erisman, Fellow, American Lung Association

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

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

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)

Mast cells are widely distributed throughout the different organs of mammals and are generally found in loose connective tissues. These cells are considered to be the origin of numerous mediators of immediate hypersensitivity. Preformed bioactive substances such as histamine, serotonin, and the peptide eosinophil chemotactic factor of anaphylaxis have been found to occur in mast cells. In the present study, immunoreactivity to selected regions of pro-ACTH/β-endorphin was found in rodent-free mast cells and mast cells in situ. Immunocytochemical localization was demonstrated for the NH_2-terminal (16K) region of pro-ACTH/β-encorphin, $ACTH^{1-24}$, $ACTH^{34-39}$, bβ-lipotropin$^{39-45}$ and β-endorphin. These findings suggest that mast cells contain a precursor with similar structural properties to that found in the pituitary gland. In addition, ACTH and β-endorphin, as measured by radioimmunoassay, were released (0.1-0.5μg/10^6 mast cells) concomitantly with histamine from free peritoneal and thoracic mast cells treated with compound 48/80, a known mast cell noncylotytic degranulating agent. Studies are in progress to confirm that peptides elaborated from mast cell pro-ACTH/β-endorphin have biological activity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Free mast cells are obtained from exsanguinated rats by lavage of the peritoneal and thoracic cavities with a balanced salt solution. Enrichment of mast cells is accomplished by centrifuging the cells through 38% (w/v) bovine serum albumin in Tyrode's solution minus Ca^{2+}. Iodination of peptide hormones of fragments utilized chloramine T as the oxidant. The labeled peptide is isolated free of reactants by passage over gel filtration columns or elution from silicic acid. Peptides in experimental samples are quantitated by radioimmunoassay (RIA) or by bioassay, e.g., endorphin-like activity is assayed by suppression of stimulated contractions of the rat vas deferens preparation. For immunohistochemical localization of peptides we employ the immunoperoxidase-bridge technique. Isolation and purification of peptides is accomplished by various methods that include molecular sieving, ion-exchange chromatography, high pressure liquid chromatography, sodium dodecyl sulfate gel electrophoresis, and affinity chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: (1) [Leu^5]-enkephalin-like immunoreactivity in mouse mastocytoma. The incentive to examine normal mast cells for ACTH - and opiate-like peptides originated with our observation that extracts of a mouse mastocytoma (P-815) contain material that cross-reacts with an antiserum to the opiate peptide [Leu^5]-enkephalin. This observation indicated that mast cell tumors were capable of ectopic synthesis of peptide hormones, or that the detected immunoreactivity (IR) represented a peptide normally elaborated by mast cells. Negligible [Met^5]-enkephalin-IR was detected in the extracts. Gel filtration of acid extracts of the mastocytoma cells revealed [Leu^5]-enkephalin-IR between 4000-8000 (4-8K) daltons and 2,500 daltons. No immunoreactivity was detected at the elution position of the free [Leu^5]-enkephalin pentapeptide. Treatment of the 4-8K peak material with trypsin or cyanogen bromide resulted in partial retention of the original peak and another immunoreactive product at about 1K. Further analyses of the [Leu^5]-enkephalin-IR substance is still in progress. Our preliminary studies indicate that normal mast cells do not contain [Leu^5]-enkephalin, which suggests that this peptide is an ectopic product elaborated only by the mastocytoma.

(2) Evidence for Sequences of Pro-Corticotropin/β-endorphin in Rat Mast Cells. Using various antisera, we examined normal rat mast cells by immunocytochemical localization techniques for the presence of peptides related to the known opiate peptides. Initially, we were encouraged by the finding that both $ACTH^{1-24}$- and β-endorphin-immunoreactivities were present in these cells. Staining occurred in free peritoneal-thoracic mast cells and tissue mast cells (in situ). Since these peptides are known to originate from a common precursor (pro-ACTH/β-endorphin), we examined mast cells for immunocytochemical localization of other sequences in this protein. Antisera to the NH_2-terminal (16K) region of pro-ACTH/β-endorphin, $b\beta\text{-lipotropin}^{39-45}$, and $ACTH^{34-39}$ also revealed immunolocalization in mast cells. In each case, a granular cytoplasmic staining pattern was observed and nuclei were not stained. Antisera to bradykinin, neurotensin, substance P, growth hormone, and prolactin

gave negative immunostaining. Comparison of immunoassayable ACTH in extracts of peritoneal-thoracic cells revealed that the specific activity (ACTH-IR/10^6 cells) increased with purification of mast cells.

Treatment of free mast cells in vitro with the histamine liberator compound 48/80 yielded significant amounts (0.1-0.5µg/10^6 mast cells) of immunoreactive equivalents of ACTH and β-endorphin in the medium. In each case, the known material produced a parallel displacement relative to the synthetic standard curve in the RIA. By subjecting mast cells to different conditions that cause variable amounts of histamine to be released in response to 48/80, we observed that there was a concomitant release of immuno-reactivity to ACTH or β-endorphin and histamine. These findings suggest that such peptides are stored and released in the same manner as known preformed mast cell mediators. Other investigators have reported that a peptide with vasoactive intestinal polypeptide-IR exists in mast cells and can be released under conditions similar to those reported herein. There are earlier reports that suggest that more than one peptide hormone from separate precursors can exist within the same cell. For instance, we found two opiate-like peptides, [Met^5]- and [Leu^5]-enkephalin, which are considered to originate from separate precursors, in the same adrenal medullary cell.

Further studies are now in progress to characterize and confirm the presence of pro-ACTH/β-endorphin peptides in mast cells. The apparent high levels of these peptides released from mast cells should allow for compositional analyses and bioassays. We propose to examine mast cell endorphin-IR for its potential to exhibit opiate-like activity in the isolated rat vas deferens preparation. In other proposed collaborative studies we intend to examine the capacity of mast cells to synthesize de novo ACTH using incorporation of labeled amino acids into the peptide or using in situ hybridization of mast cells with a labeled DNA probe for regions of pro-ACTH/β-endorphin-like immunoreactivity have chemical identity to the corresponding peptides in the pituitary gland. Since sequences of the heavy chain of human IgG_1 have been reported to have regions with partial homology to ACTH and β-endorphin, it will be improtant to rule out the presence of this protein or its fragments to account for these pituitary peptides in mast cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is now well documented that mast cells contain and release numerous highly active pharmacological substances. Mast cells situated in or near epithelia of the lung and gastrointestinal tract and exposed to certain antigens or basic compounds can exert profound physiological effects on these organs. The presence of several diverse peptide and non-peptide mediators indicate that these cells have the potential for controlling various cellular functions following stimuli that evoke degranulation. Some of these mediators have been implicated in the excessive mucus discharge, bronchoconstriction, or edema of the lungs in response to airborne pollutants. By understanding the chemical nature of these mediators, we can begin to assess the particular local or systemic functions such substances have in normal or pathological conditions. This information should provide a rationale for (1) implicating mast cells in tissue responses

that are beneficial or detrimental to the host and (2) assessing the potential of various compounds to modulate secretion of mast cells or the effects of mediators.

PUBLICATIONS

Linnoila, R.I., Abe, T., Voytek, P. and DiAugustine, R.P.: Coupling of amines to and cross-linking of endogenous eytosol or membrane proteins by hepatic transglutamenase. Biochem. Pharmacol. 28: 1601-1608, 1979.

Kahn, M.N., Lazarus, L.H., Mirel, R.D., Ontges, D., Ghosh, A.P., and DiAugustine, R.P.: Evaluation of extreme carboxy terminal ($ACTH^{34-39}$) and solid-phase radioimmunoassays of adrenocortiocotropin. Endocrinol. (in press).

DiAugustine, R.P., Lazarus, L. H., Jahnke, G., and Erisman, M.D.: β-endorphin-like immunoreactivity in mast cells, Pharmacology Symposium, University of Iowa, August 15-16, 1980, Life Sciences (in press).

Linnoila, R.I., DiAugustine, R.P., Hervonen, A. and Miller, R.J.: Distribution of [Met^5]- and [Leu^5]-enkephalin-, VIP, and substance P-like immunoreactivies in human adrenal glands. Neuroscience (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25006-03 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on 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
Others:	B. E. Tainer	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.20	0.30

CHECK 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 unstable bioregulator thromboxane A_2 (TXA_2) is generated from the prostaglandin (PG) endoperoxide PGH_2 by an enzyme called thromboxane synthetase. Thromboxane synthetase is present in many tissues including blood platelets, lung, spleen, and brain. Simultaneous with the formation of TXA_2 is the formation of C-17 hydroxy fatty acid (HHT) from PGH_2. We have investigated this process with emphasis on kinetics and inhibitors. We have shown that HHT (12-Hydroxy-5,8,11-hepadecenoic acid) is not a breakdown product of TXA_2 but that TXA_2 breaks down exclusively into thromboxane B_2. Moreover, our results indicate that thromboxane synthetase is not an isomerase but rather that a biomolecular reaction is involved in the formation of TXA_2 and perhaps HHT. Also, we have preliminary evidence that there is another unstable intermediate formed from PGH_2 which breaks down to HHT. The existence of another intermediate poses interesting experimental questions concerning possible biochemical and biological activities.

PHS-1040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Ram seminal vesicle microsomes were used to biosynthesize ^{14}C-PCH$_2$ or ^{3}H-arachidonic acid. Washed human platelets and human platelet microsomes were prepared from platelets obtained from the Red Cross. The conversion of PGH$_2$ to TXB$_2$, (the stable endproduct of TXA$_2$) and HHT (12L-hydroxy-5,8,10-heptadecaenoic acid) were measured using thin layer or high pressure liquid chromatography. TXA$_2$ was trapped as the O-methyl derivative and measured on TLC or HPLC. The in situ formation of HHT was followed by measuring the absorbance of HHT at 236 nm using a DW-2-spectrophotometer.

MAJOR FINDINGS AND PROPOSED COURSE: Our data and reports in the literature show that TXB$_2$ and HHT are produced in approximately the same amounts when AA or PGH$_2$ are incubated with thromboxane synthesizing tissues. Inhibition of thromboxane synthetase appeared to inhibit the production of both TXB$_2$ and HHT to the same extent. One possible explanation of these observations is that TXA$_2$ could break down into either TXB$_2$ or HHT. However, our results explicitly show that HHT is not a breakdown product of TXA$_2$ but rather TXA$_2$ decomposes exclusively into TXB$_2$.

A model for the enzymatic generation of TXB$_2$ and HHT from PGH$_2$ has been proposed which is consistent with the above observation. It was suggested that PGH$_2$ can rearrange into either TXA or HHT and malondialdehyde. Our enzyme kinetic results are consistent with this model. The rate of formation of TXB$_2$ is clearly dependent upon [PGH$_2$]^2 and thus a biomolecular reaction is involved in the formation of TXB$_2$. A reaction mechanism in which one molecule each of TXA$_2$ and HHT are produced simultaneously from two molecules of PGH$_2$ is consistent with our kinetic results as well as the above-mentioned observations.

In preliminary experiments, we were able to measure the formation of HHT by following its absorbance of 236 nm. The time course for the formation of a PGH$_2$ metabolite (in situ) absorbing after extraction and analysis by TLC. Thromboxane synthetase inhibitor inhibits the in situ formation of a 236 nm absorbing metabolite. These preliminary observations indicate that PGH$_2$ is converted to an unstable intermediate which breaks down during isolation to HHT.

Comparison of HHT formed in situ to that formed as measured by TLC was complicated by (a) non-enzymatic production of HHT during the work-up and (b) accurate measurement of HHT absorbance in turbid solutions. A method was developed that quantitatively reduces unreacted PGH$_2$ to PGH$_{2\alpha}$, thus preventing non-enzymatic HHT formation. However poor TLC separation necessitated the development of a HPLC (see project on Pulmonary Biosynthesis) method to achieve good separation. Methods were also developed for the solubilization and partial purification of thromboxane synthetase from platelet microsomes. The use of solubilized enzyme preparations appears to permit accurate measurement of in situ to obtain better and more complete evidence for the existence of a new unstable intermediate. Thus, thromboxane synthetase appears to form two unstable intermediates, one of which (TXA$_2$) breaks down to TXB$_2$, the other to HHT. We plan to further investigate thromboxane synthetase and acquire additional evidence to support

our hypothesis of an HHT intermediate. We propose to isolate or trap this unknown, characterize its biological activity and determine the mechanism of its formation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Thromboxane synthetase is present in many tissues including lung, blood platelets, spleen and brain. Thromboxanes have very potent effects on smooth muscle including that of lung airways. They have been implicated as primary mediators in platelet aggregation. A variety of stimuli can release significant amounts of thromboxanes from the lung. Since prostaglandins and thromboxanes are stored in tissues, understanding the factors that control and regulate their biosynthesis is most important in understanding the physiological role of these bioactive substances. The possible discovery of an additional unstable intermediate in prostaglandin biosynthesis with unknown biological activity may open new areas for the role of PG in biological processes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25007-02 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
NIH, NIEHS, 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)

Chrysotile asbestos clearly is cytotoxic and has been implicated as an etiologic factor in asbestosis and neoplasia in man and experimental animals. Very little is known concerning the early events of particle translocation and cell injury which lead to the characteristic diseases. To determine the initial impaction site and clearance pathways of inhaled fibers, rats were exposed to 4 mg/m^3 (respirable mass) of chrysotile asbestos for 1 hour. Animals were anesthetized immediately after each exposure, the tracheas were clamped, and the lungs perfused with Karnovsky's fixative through the right ventricle. Blocks of tissue were prepared for light, transmission and scanning electron microscopy.

We have shown that inhaled asbestos impacts initially on alveolar duct bifurcations and that significant interactions take place between the asbestos and underlying alveolar duct epithelial cells. Early clearance through the alveolar epithelium could be a highly significant mechanism for introducing small asbestos fibrils into the pulmonary interstitium where interactions with macrophages and fibroblasts are known to occur during the pathogenesis of asbestos.

PROJECT DESCRIPTION

METHODS EMPLOYED: Groups of three 8 week old male rats were exposed in inhalation chambers to chrysotile asbestos. The chambers were designed according to the well-established models of Wagner and Skidmore (Wales, United Kingdom). The chrysotile is a Canadian asbestos with great variability in fiber length and diameter, as expected for a fibrous serpentine. Previous studies in this laboratory have shown that the elemental ratio of Mg to Si is about .70 $\pm$ .04 (Brody, 1980) in the aerosolized chrysotile.

Rats were exposed while held individually in plexiglass cylinders. This allowed a nose only exposure to a respirable mass of 4.0 mg per m^3 $\pm$ .3 mg/m^3 of chrysotile. Each animal was exposed for 1 hour. Immediately following exposure, animals were removed from the inhalation chamber and anesthetized by an intraperitoneal injection of Nembutal (1 mil of a 50 mg/mil solution). Additional exposed animals were placed individually in open cages with food and water and then sacrificed at 5 hours, 24 hours, 4 days and 8 days after cessation of exposure. As soon as the anesthetized animal is insensitive, the trachea is exposed and clamped. After opening the chest wall, 0.85% saline and 30 seconds later, buffered Karnovsky's fixative are perfused through the right ventricle and pulmonary artery. Tissue blocks are dissected from five predetermined sites and are prepared for light and electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Scanning electron microscopy was used to quantitate the degree of asbestos deposition on alveolar duct and alveolar space surfaces. Our data show that the vast majority of particles inhaled beyond terminal bronchioles impact initially upon bifurcations of alveolar ducts The farther the alveolar duct bifurcation is from its bronchiole, the less asbestos is observed. Duct bifurcations in the lungs of animals recovering in room air for 5 hours clearly exhibit less asbestos than observed immediately after 1 hour exposure. It is unlikely that these fibers were cleared by macrophages because the number of alveolar phagocytes was not increased. We learned by transmission of electron microscopy that small asbestos fibrils were taken into the cytoplasm of underlying Type I epithelial cells and alveolar-capillary basement membranes within the first hour of dust exposure.

We propose to determine the role of alveolar epithelial cells in transporting inhaled asbestos from the air spaces to pulmonary interstitium. Such a clearance pathway could be highly significant for introducing small fibrils into the connective tissue compartment where interactions with macrophages and fibroblasts are known to occur. In addition, we have preliminary evidence that a 1 hour exposure to chrysotile asbestos causes a lesion in alveolar duct walls. Morphometric studies are ongoing to establish whether or not this is true.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Asbestos inhalation clearly causes severe pulmonary disease. We are attempting to understand the earliest events leading to expression of the characteristic fibrotic response. Particle deposition and translocation, particle-cell inter-

actions, macrophage response and cell injury are essential parameters yet to be fully defined. Elucidation of these events are likely to move us closer to a more complete comprehension of asbestos induced lung disease.

PUBLICATIONS

Brody, A. R., Hill, L. H.: Deposition pattern and clearance pathways of inhaled chrysotile asbestos. Chest (in press), 1980.

Crapo, J.D., Brody, A.R., et. al.: Morphometric and X-ray microanalytical studies on lung tissue of rats exposed to chrysotile asbestos. Proc. IARC Symp. on Biol. Effects of Min. Fibers (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25008-02 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
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INSTITUTE AND LOCATION
NIH, NIEHS, 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)

Chrysotile is the most commonly used asbestos mineral, and it clearly is the most cytotoxic, both in vitro and in vivo. There is good evidence that the elemental composition of chrysotile contributes to its cytotoxic nature. We have studied the translocation of Mg and Si ions from chrysotile fibers under the following experimental conditions: (1) After treatment with dehydrating agents, fixatives and embedding media, (2) After treatment with 1NHCL for 16 hours, (3) Following phagocytosis by alveolar macrophages, (4) After inhalation by rats.

Transmission and scanning electron microscopy in concert with X-ray energy spectometry showed that significant amounts of Mg had slowly leached from asbestos fibers after one month in 2.5% glutaraldehyde.

Treatment with 1NHCL caused a rapid (one hour) loss of significant Mg content which reached a plateau at about 4 hours. In contrast, two hours after phagocytosis by alveolar macrophages, no Mg loss was detected. Chrysotile fibers in the lungs of rats showed considerable variability in Mg content.

PROJECT DESCRIPTION

METHODS EMPLOYED: Transmission and scanning electron microscopy in concert with X-ray energy spectrometry were employed to determine the elemental content of individual asbestos fibers. This was accomplished through detection and recording of X-rays specific for Mg and Si. After 100 sec. counting time, a ratio of the number of X-rays collected was calculated by dividing the number of data points used to determine the degree of elemental change occurring in chrysotile asbestos under a variety of experimental conditions.

Since tissues from animals inhaling asbestos and cells phagocytizing asbestos must be fixed, dehydrated and embedded, we treated chrysotile asbestos with a solution of 2.5% glutaraldehyde, phosphate buffer; 1% osmium tetroxide, 100% ethyl alcohol, propylene oxide and Epon. Additional samples of untreated fibers were analyzed to establish control Mg/Si ratios. Other samples were treated with 1NHCL over a 16 hour period to provide a positive control of our ability to detect changes in Mg content of individual fibers.

Alveolar macrophages lavaged from the lungs of rats were plated in monolayer cultures for 1 hour and then exposed to chrysotile asbestos in the culture medium. Groups of rats were exposed in carefully monitored inhalation chambers to aerosolized chrysotile asbestos at a respirable mass of $4mg/m^3$. Cells and tissues from these experiments were fixed, dehydrated and embedded by conventional techniques for light and electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: Previously we had established that the Mg/Si ratio of unaltered chrysotile asbestos is .70 $\pm$ .04(1SD). Only after one month in 2.5% glutaraldehyde was the elemental ratio significantly changed to .64 $\pm$ .04. Other reagents such as buffers and alcohols had no effect within the times tested. After two hours in alveolar macrophage cultures, phagocytized asbestos showed no detectable change in elemental content. In contrast, inhaled particles showed a wide range of alteration in Mg/Si ratio. For example, interstitial fibers in both macrophages and connective tissues exhibited ratios from .40 to control levels. Since these animals had been inhaling asbestos continuously for three months, it was not possible to know how long the interstitial fibers had been residing in the lung. However, we were able to establish that a subpopulation of platy serpentine particles was predominant in the alveolar spaces of these animals. This was determined through calculation of a low Mg/Si ratio (.30 $\pm$ .03) characterizing these particles as talc.

Short-term inhalation experiments are underway so we will be able to precisely define the residence time of asbestos in the lung. Macrophages and the a cellular alveolar lining layer lavaged from these animals will be studied to assess the effects of control and Mg-leached asbestos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The elemental content of inhalable inorganic particulates may play an important role in cytotoxic events leading to pulmonary disease. There is substantial

evidence that the size and shape of asbestos fibers also is critical in this regard. The question of which factors are most important clearly is open and requires much additional work. We intend to pursue such questions through *in vitro* experiments utilizing pulmonary cells such as alveolar macrophages and through animal inhalation studies where cytotoxic dusts can be investigated *in situ*.

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. (in press), 1980.

Crapo, J.D., Brody, A.R., *et. al.*: Morphologic, morphometric and X-ray micro-analytical studies on lung tissue of rats exposed to chrysotile asbestos. Proc. IARC Symp. on Biol. Effects on Min. Fibers (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25009-01 LPFT

PERIOD COVERED
October 1, 1979 to September 30. 1980

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

Sialoglycoproteins (SG) on the surface of rat red blood cells (RBC) were labeled with gold-conjugated wheat germ agglutinen (WGA). Before labeling, RBC's were treated with aliquots of chrysotile asbestos. Asbestos fibers firmly adhere to red cell surfaces. A decrease in the number of SG-WGA compelxes was observed on the surface of asbestos treated RBC's. It is proposed that positively charged Mg ions in the chrysotile fibers bind to negatively charged sialic acid molecules on the red cell surface. This interaction leads to cell distortion, loss of some membrane components, consequent translocation of intracellular ions and finally, hemolysis. The hypothesis is being tested in vitro with morphologic and biochemical techniques. These methods will be applied to pulmonary components such as macrophages and epithelial cells which are known to interact with inhaled chrysotile asbestos.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Red blood cells (RBC's) were collected from CD rats. Immediately after collection, the RBC's were diluted in .85% saline to a 2% (vol.) solution in 15 ml. centrifuge tubes. All control and asbestos reactions were carried out at 37°C.

Gold spheres (prepared in our laboratory from AuCl) measuring .05 μm were complexed with commercially prepared wheat germ agglutinin (WGA) by established methods using glutaraldehyde. WGA-Au complexes were used to specifically label sialoglycoproteins on the surface of RBC's. The following experiments were carried out:

1) RBC's no treatment of any kind
2) RBC's asbestos (4 hours)
3) RBC's WGA-Au or RBC Au spheres
4) RBC's asbestos subsequent WGA-Au label
5) RBC's WGA-Au subsequent asbestos

All specimens were fixed in 2.5% glutaraldehyde following the experiment, dehydrated in ethyl alcohols and then critical point dried for scanning electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: RBC's labeled with WGA-Au exhibited a dense even surface distribution of the complexes. Gold spheres alone rarely attached to the cell surface. Asbestos treated RBC's usually were distorted and asbestos fibers adhered tightly to the cell surfaces. The WGA-Au label on these cells was sparse and unevenly distributed over the cell surfaces. Interestingly enough, RBC's treated initially with WGA-Au label did not interact significantly with the asbestos, suggesting that the RBC-asbestos binding was blocked by the presence of the WGA-Au complex.

These studies further substantiate the hypothesis that positively charged Mg ions in asbestos fibers bind to negatively charged sialic acid molecules on red cell membranes. This interaction purportedly leads to redistribution of membrane components and subsequent cell death by translocation of intracellular ions. We are pursuing this hypothesis further by quantifying sialic acid with biochemical and further morpholigical techniques, by analyzing ion shifts of RBC's during asbestos treatment, by studies of neuraminidase treated RBC's and be using the surface labeling techniques to study hemolysis caused by non-asbestiform minerals. Subsequently, we will test the hypothesis on other pulmonary components such as macrophages and epithelial cells which are known to interact with inhaled chrysotile asbestos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cytotoxic reactions are major elements in the pathogenesis of dust-related pulmonary diseases. These studies attempt to explain a basic biologic mechanism of membrane injury leading to cell death. The fact that a commonly used asbestiform mineral (chrysotile) causes such an injury *in vitro* and is likely

to do so *in vivo* after inhalation, is a major issue being pursued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25011-01 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	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)

It is the long range goal of this project to determine whether tumor promotion by various agents, e.g., phorbol esters, proceeds via the release of arachidonic acid (AA) and the subsequent synthesis of prostaglandins (PG) by hydroxy fatty acid (HFA). We have examined several fibroblasts and chosen the mouse embryo fibroblast (10T 1/2) as a model system. PG production by these cells was examined with respect to cell growth and cell density. Phorbol esters (TPA) altered cell growth and PG + HFA production. Our goal is to differentiate between the release of AA, formation of PGs and HFAs by use of selective inhibitors of these pathways.

PHS-6040
(Rev. 10-76)

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 Pgs, TXs, and HFAs 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 made by these cells and the ability of TPA to promote 10T 1/2 cell transformation. Production of PG by these cells was characterized; the amount of PG produced was highly dependent on cell density and growth.

On incubation of ^{3}H-AA for 24 hours, approximately 25% of ^{3}H-AA was incorporated into the cells. The addition of 10^{-7} to 10^{-4}M TPA produced a 3-fold stimulation of AA release over a 24 hour period. Maximum stimulation was observed at approximately 3-4 hours. Release was similar in log growing and confluent cells but more PG, and HFA were detectable in the media from cells at confluency. TPA at a concentration of 10^{-5}M produced maximum stimulation of release. With these basic experiments finished we are presently investigating the effects of various inhibitors in the release of AA and formation of PGs and HFA. The following inhibitors will be used: (a) nonsteroidal anti-inflammatory agents; indomethacin, phenylbutazone, block formation of PGs only, (b) steroids; hydrocortisone dexamethasone trianicin-alone-blocks release of AA, (c) ETYA-blocks formation of both PGs and HFA, (d) lipoxidase inhibitors; BW-755C, -blocks formation of HFA. We are also characterizing the HFA formation by 10T 1/2 cells. After obtaining data on the concentration and time of exposure to these inhibitors, we intend to study the effect of these agents on the promotion of benzo(a)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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25012-01 LPFT

PERIOD COVERED

October 1, 1979 to September 30, 1980

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:	A Ali	Visiting Fellow	LPFT	NIEHS
Others:	T. Eling	Head, PG	LPFT	NIEHS
	C. Barrett	Head, ECG	LPFT	NIEHS
	J. Boyd	Technician	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Prostaglandin Group

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	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 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; (2) characterize the events which inhibit or promote platelet aggregation in vivo; (3) compare the response of pulmonary artery and pulmonary vein to stimuli which enhance the metabolism of fatty acids in vivo; (4) the mechanism(s) of action of chemicals and environmental agents (to be screened) which may stimulate intra-arterial thrombosis; (5) determine the mode of secretion and transport of pulmonary prostaglandins and thromboxanes via capillary endothelium.

PHS-3040

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: A few cell lines other than those of endothelial origin can produce prostacyclin (the most potent anti-aggregatory)a mouse embryo fibroblast line) and SHE cell lines also produce PGE_2 and $PGF_{2\alpha}$ prostacyclin was also unidentifiable. Cells from bovine aorta endothelium (ABAE) as well as bovine fetal heart endothelium (FBHE) were also tested for lines produced predominantly PGI_2 (prostacyclin) as the main product of arachidonic acid metabolism while they lack the ability to produce thromboxane A_2 (monitored by measuring its stable metaboliate TXB_2). On incubation of these lines (confluent monolayers with 20mM sodium arachidonate in Hepes buffer for 2 minutes at 37°C) the characteristic activity of prostacyclin as inhibitor prevented by incubation of cells in media containing indomethacin or tranylcypromine (a PGI_2-synthetase inhibitor). However, a significant difference was observed between the different cell lines in that they produce varying amounts of prostacyclin-like activity depending on the tissue of origin. Thus ABAE cells were about 192% and 364% as active as FBHE and 10T 1/2 cells respectively as producer of PGI_2.

ABAE endothelial cells were selected to study the uptake and release of AA and the factors that control these processes. However, during the course of the experiments the ABAE cells transformed to giant cells. A primary culture of pig aortic endothelial cells (YPAE) was developed and the synthesis of PG, TX and HFA examined. YPAE produced mainly PGI, small amounts of PGE_2 and several unknown AA metabolites that appear to be dihydroxyfatty acids. We intend to use these cells to study the factors that alter PGI_2 biosynthesis. The effect will be studied further as related to the effects of vitamin K in the blood clotting process.

The interaction between pulmonary endothelial cells and platelets was studied using theisolated perfused lung system. The appearance of TXB_2 and aggregates in perfusate was an indicator of platelet aggregation. Perfusion of rat lung with rat platelets did not result in aggregation. Inhibition of pulmonary PGI_2 biosynthesis of 15-HPAA or asa resulted in the appearance of TXB_2 in perfusate. Similar results were obtained with human platelets. The lung also makes TXB_2 but the TXB_2 observed in the perfusate originates from platelets. Perfusion of guinea pig lung with human platelets produced TXB_2.

Inhibition of PGI_2 was not required. In vitro incubation mixture of guinea pig and rat lung microsomes showed that rat lung makes larger amounts of TXB_2 with little PGI_2. Guinea pig platelets were found to be sensitive to PGI_2 when compared with rat or human platelets. There appeared to be an inverse relationship between pulmonary biosynthesis of PGI_2 and sensitivity of the platelets to inhibitory action of PGI_2. Our proposed cause is to (1) further study the interaction between endothelial cells and platelets (2) examine the effect of exposure to oxidant gases on the system.

SIGNIGICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cerebral and coronary strokes (heart attacks) as a result of intra-arterial thrombosis constitute the major cause of death in this country. Little is known of the causes and the mechanisms that control the formation of intra-arterial thrombosis. The lung with its vascular bed and extensive endothelial lining apparently play a major, yet undertermined, role in the control of platelet aggregation and thus thrombi formation. Changes in this endocrine function of pulmonary tissue by exposure to environment agents may have an impact on the state of mechanisms that control the pulmonary formation of PGI_2 which should thereby sugnificantly contribute to our understanting of and ability to prevent intra-arterial thrombosis.

PUBLICATIONS

Ali, A. E., Barret, C. J., and Eling, T. E.: Prostaglandin and thromboxane production by vascular endothelial cells and fibroblasts. Prostaglandin (in press).

Boy. J. and Eling, T. E.: Prostaglandin release and the interaction of platelets with pulmonary vasculature of rat and guinea pig. Thrombosis Research (subcommittee).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25013-01 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Biology of Non-Ciliated Bronchiolar Cells (Clara cells) in vitro

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

COOPERATING UNITS (if any)

Environmental Protection Agency (EPA), Research Triangle Park, North Carolina

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:
0.5	0.5	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)

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 investigate the function of Clara cells by developing in vitro cell and organ culture models with which to examine factors and mechanisms that induce and control Clara cell proliferation, differentiation and secretion.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

Our objectives are to study the factors and mechanisms that induce and control bronchiolar Clara cell proliferation, differentiation and secretion. To facilitate these studies, we are developing in vitro cell and organ culture systems with which we can examine the biology of bronchiolar epithelium. Our research plan is, (1) to define the minimal cell and organ culture conditions necessary for maintenance of viable bronchiolar epithelium, (2) to define the specific culture conditions necessary for proliferation and determine the hormones required for differentiation and/or proliferation, and (3) to determine the influence of extracellular matrix components on airway cell growth and differentiation. Baseline steady-state parameters for bronchiolar epithelium will be determined from whole lung and isolated airways.

METHODS EMPLOYED:

Procedures have been developed to aseptically dissect intact airways including terminal bronchioles from whole rabbit lung lobes. Explants are prepared from the isolated bronchiolar structures and placed in culture in a semi-defined medium containing insulin (5µg/ml) and hydrocortizone (1µg/ml) on cellulose rafts or collagen substrates. Minced, isolated bronchioles are sequentially digested with 0.1% collagenase to provide viable epithelial cells for primary cell culture. We are pursuing the kind of culture conditions which are necessary for maintenance, proliferation and/or differentation of the bronchiolar cells and organ cultures. Particular attention is being paid to synthetic media, serum supplementation, 3T3 conditioned medium, growth hormone factors and extracellular matrix components. Specific embedding and sectioning procedures have been developed so explant epithelium can be assessed morphometrically as well as qualitatively. The cellular composition, viability and ultrastructural integrity of explants and cell cultures are assessed by light and transmission microscopy at different times in culture under different conditions. The cellular response to in vitro conditions in terms of proliferation and differentiation are determined morphologically and by histochemistry, autoradiography and morphometry.

MAJOR FINDINGS AND PROPOSED COURSE:

These studies are currently underway; quantitative data are not yet available able. The majority of explants of terminal bronchioles were viable up to 10 days in TC medium 199 and Waymouths MB 752/1 supplemented with 10% fetal bovine serum, insulin and hydrocortisone. Explanted airways consisting of preterminal and terminal bronchioles cultured on or in collagen gels for 7 days underwent proliferation and differentiation. Epithelial cell mono-layer outgrowths developed from the cut and dissected ends of airways. The cultured airway mucosa consisted mostly of morphologically distinct ciliated cells and Clara cells. The apical cytoplasm of Clara cells bulged into the lumen beyond the ciliated cells and contained numerous secretory granules. Dissociated bronchiolar cells were plated at a density of 5×10^5/ml on collagen and fibronectin (5mg/ml) coated coverslips in 1:1 F12K-3T3 conditioned Dulbecco's Minimal Essential Medium. The cultures became confluent in 5 days in

DMEM supplemented with insulin (5mg/ml), transferrin (5mg/ml), progesterone (2×10^{-8}m), selenium (3×10^{-8}m), LRF (10 ng/ml), FSH (0.4 mg/ml) and EGF (5 ng/ml). To subculture, coverslips from primary cell cultures were broken into pieces and placed in 35 mm collagen-fibronectin-coated dishes containing culture medium. The subcultured cells were confluent after approximately a month at which time the cells were trypsinized and subcultured to collagen-fibronectin-coated 25cm^2 flasks where they demonstrated a high attachment frequency. These cells had a low attachment frequency in non-treated flasks or flasks coated with serum. We conclude that collagen and fibronectin facilitate the establishment of bronchiolar cells in vitro and may be a requirement for their subculturing. The ultrastructure, staining characteristics and passage ability of these cells is being studied.

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.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80008-06 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biosynthesis, Release, Transport and Metabolism of Prostaglandins [PGs] 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

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
Other:	R. Warnock	Intermittent	LPFT	NIEHS
	J. Boyd	Temporary Technician	LPFT	NIEHS
	B. Tainer	Biologist	LPFT	NIEHS
	A. Ali	Visiting Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology and Chemistry Branch, Duke University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	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 long-range goal of this project 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. Topics of current interest are the structural requirements of prostaglandins needed for transport, the effect of oxidant gases on transport, and the potentiation of inflammatory reaction due to an inhibition of the pulmonary inactivation of prostaglandins. Finally, the effects that exposure to environmental agents has on the biosynthesis of Prostaglandins, Thromboxanes and Hydroxy fatty acids in the lung and the release of these agents from the lung is being investigated.

PHS-6040
(Rev. 10-76)

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 (PGs) and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography 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: We have studied the effect of exposure of rats and guinea pigs to 100% O_2 and NO_2 PG biosynthesis and metabolism by the lung. O_2 and NO_2 significantly depressed the enzyme system that metabolizes PG to inactive metabolites (Prostaglandin dehydrogenase PDGH) but is without effect on prostaglandin synthetase or the transport carriers. After exposure to either NO_2 or O_2, depressed PGDH returned to normal levels within 5 days. Kinetic studies indicate noncompetitive inhibition of PGDH on exposure to O_2 which is consistent with destruction of the enzyme by O_2 gas.

We have investigated the potentiation of anaphylaxis in guinea pigs by exposure to 100% O_2. Guinea pigs sensitized to albumin were exposed to 100% O_2 for 72 hrs, the lung isolated and perfused. The perfusate during anaphylaxis was collected and analyzed for vasoactive material. The major prostaglandin (60%) released during anaphylaxis was TXB_2 with lesser amounts of $PGF_{2\alpha}$, PGE_2, 15-keto 13,14-dihydro $PGF_{2\alpha}$. These PG were detected and estimated by RIA. Exposure to 100% O_2 increased the TXB_2 release 2-3 times, elevated $PGF_{2\alpha}$, PGF_2 and 6-keto $PGF_{1\alpha}$ release but decreased the release of the $PGF_{2\alpha}$ metabolite 15-keto 13,14-dihydro $PGF_{2\alpha}$. Histamine release, which is also an indication of the degree of anaphylaxis, was unchanged. The exposure to 100% O_2 appears to potentiate the release of TX and PG during anaphylaxis by inhibition of their metabolism. This may be in part responsible for the potentiation of systemic anaphylaxis observed after exposure to O_2 and other oxidant gases.

The lack of rapid and reliable methods that permit good separation of Pg, TX and HFA has severely hindered biosynthesis studies. We have developed an HPLC method that gives rapid and good separation of the known metabolites of AA. Using a reverse phase column, isocratic elution separates PGE_2, PGF_2,

TXB_2 and 6-keto $PGF_{1\alpha}$. Further elution by a gradient permits separation of HHT, HETE's and AA. Thus, all AA metabolites can be separated during a single chromatographic run. The use of a radial compression column permits the use of high flow rates that decrease the elution time without affecting the separation of the AA metabolites. We have used this system to study PG and TX formation by rat, guinea pig, bovine and porcine pulmonary tissue. Our results show that rat makes mainly PGI_2 and little TXB_2, while pig and beef lung make approximately equal amounts of PGI_2 and TXB_2. Guinea pig lung makes high amounts of TXB_2 with little formation of PGI_2. We intend to use this system to examine PG biosynthesis in human lung. The proposed course is as follows: 1) To examine the effect of exposure of rats to air-borne pollutants on the PG system, 2) To use isolated cell systems to examine the mechanisms for the transport of PG into and out of cells and apply the information obtained from this study to the intact lung system, 3) To examine the effect of exposure of animals to pollutants on the inflammatory release of vasoactive chemicals from the lung, 4) To measure the PG and TX obtained from human lung with pulmonary disease and attempt to correlate these findings with the disease, and 5) To study PG and TX biosynthesis in isolated lung cells with emphasis on pulmonary macrophages.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs and HFA have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes. The lung is an important site for the synthesis and metabolism of PGs; alterations in 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

Anderson, M. W., Chaudhari, A., Crutchley, D., Wilson, A. G. E., and Eling, T. E.: Studies on the covalent binding of an intermediate(s) in prostaglandin biosynthesis to various tissue proteins. Biochem. Biophys. Acta. 573:40-50, 1979.

Chaudhari, A., Eling, T. E., and Anderson, M. W.: Inhibition of pulmonary prostaglandin metabolism by exposure of animals to oxygen or nitrogen dioxide. Biochemical J. 184:51-57, 1979.

Wilson, A. G. E., Kung, A., Anderson, M. W., and Eling, T. E.: Covalent binding of arachidonic acid metabolites in platelets. Prostaglandin, 18:409-422, 1979.

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. In Press.

Eling, T., Warnock, R., Dick, D., and Tainer, B.: Separation of PG, TX, HFA and arachidonic acid by high pressure liquid chromatography. Submitted to Anal. Biochem.

Porter, N., Byers, J. P., Ali, A. E. and Eling, T. E.: Synthesis of PGG_2. J. Am. Chem. Soc. 102:1183-1186, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE

PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 ES 80023-07 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	C.T. Post	Visiting Fellow	LPFT	NIEHS
	J.W. Spalding	Research Giologist	LPFT	NIEHS
	L.B. Gilmore	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)
Laboratory of Environment of Biophysics

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (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 origins of several extracellular enzymes present in the lining.

The objectives of this investigation are as follows: (1) to elucidate the composition of the acellular lining; (2) to identify processes by which these components arise in the acellular lining; (3) to identify the subcellular processes involved in the formation of acellular lining components synthesized by the lungs; (4) to identify variations in the acellular lining of diseased and damaged lungs.

PHS-2040
(Rev. 10-76)

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 2 cells are isolated on discontinuous sucrose gradients using differential contrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis. Cadmium is measured using an atomic absorption spectrometer.

MAJOR FINDINGS AND PROPOSED COURSE: Lamellar bodies of the alveolar Type 2 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. Although secreted, the lamellar bodies contain an enzyme complement very similar to that of the lysosomes. Similarities extend to individual isoenzymes; for example, both A and B forms of β-N-acetylglucosaminidase are present in lamellar bodies and lysosomes although the ratio between the forms differ (A:B = 16:1 in lamellar bodies and 35:1 in lysosomes). Using detergent action and phospholipase C which disrupts lamellar bodies we have demonstrated that both β-N-acetylglucosaminidase and acid phosphatase are distributed throughout the lamellar body structure. However, these two enzymes occupy different sites within the lamellae. β-N-acetylglucosaminidase appears to be associated with phosphatidylcholine molecules within the structure but acid phosphatase is not.

A new method has been developed for the isolation of lamellar bodies from homogenized lungs. The method involving the use of discontinuous sucrose gradients results in a highly pure and morphologically-intact product. As indicated using marker enzymes the lamellar body preparations were free of mitochondria but were contaminated with approximately 15% microsomal protein. Membrane properties have been studied using electron paramagnetic resonance with 5- doxylstearate as a probe. Preliminary studies indicate that the membranes of the lamellar bodies are highly fluid, a property which appears to be conferred on the structures by the minor phospholipid constituents such as phosphatidylglycerol.

Lamellar bodies will be further characterized as to their composition and properties.

The effects of cadmium on the composition and formation of the acellular lining is being studied. Instillation of cadmium into the lungs results in the induced synthesis of a cadmium-binding protein which resembles metallothionein. Small amounts of this protein may be present in the acellular hydrolase content of the acellular lining appears to be elevated in response to cadmium instillation which may reflect tissue damage. These studies will attempt to define a protective role for the acellular lining against the toxic action of inhaled cadmium.

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

Spalding, J. W. and Hook, G. E. R.: Phospholipid exchange between subcellular organelles of rabbit lung. Lipids 14:606-613, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80029-04 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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:	S.E. Fabro	Consultant	LET	NIEHS
	L. B. Gilmore	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)
Department of Medicine, Duke University; Department of Gynecology and Obstetrics, George Washington University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology Group

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)

This project is concerned with the identification and characterization of components of the lung associated with pulmonary diseases which could provide information concerning the disease processes as well as function as diagnostic markers of pulmonary damage or disease. Current attention has been focused on the multi-lamellated myelin-like structures which accumulate in the alveoli and airways of patients with pulmonary alveolar proteinosis. The objectives of the study have been to elucidate the composition, structure and origins of this unusual material.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Bronchoalveolar lavage effluents from patients with pulmonary alveolar proteinosis were supplied by the Department of Medicine 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 myelin-like structures (MS) which appear to form extracellularly in the alveoli and airways of patients with this disease. Those MS consists of trilaminated membranes (85Å wide) separated by amorphous material; the distance between membranes is approximately 170Å. These structures can assume a wide variety of shapes and sizes. The basic myelin-like structure resembles the tubular myelin structure found in the alveoli and airways of normal human lungs. However, the structures from diseased lungs show numerous abnormalities involving organization and size. The membranes are probably lipid since they can be extracted with chloroform/methanol (2:1). The amorphous material between the membranes is protein as indicated by its susceptibility to digestion by trypsin.

The myelin-like structures from the lungs of patients with alveolar proteinosis will be further investigated using chaotropic agents, detergents, lipases and proteases. Attempts will be made to isolate the structures and determine their composition.

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 on the myelin-like material which accumulates in the lungs of patients with pulmonary alveolar proteinosis indicate that the material may be derived from tubular myelin a normal constituent of the lungs. The disease appears to involve abnormal assembly of the tubular myelin structure.

PUBLICATIONS

Bell, D.Y. and Hook, G.E.R.: Pulmonary alveolar proteinosis: analysis of airway and alveolar proteins. Am. Rev. Resp. Dis. 119: 979-990, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-04 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Synthesis of Polypeptide Hormones and Prohormones by Normal and Neoplastic Lung Epithelium

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:	L. H. Lazarus	Research Chemist	LPFT	NIEHS
	I. Linnoila	Visiting Associate	LPFT	NIEHS
	P. Nettesheim	Chief	LPFT	NIEHS
	E. Sisskin	Staff Fellow	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)
M. D. Erisman, Post-doctoral Fellow, Duke University, Durham, NC
Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

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

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 lung epithelium of mammals is known to contain rare solitary neuroendocrine-like cells. Presently, we have not been able to identify any known polypeptide hormones in these cells by immunohystochemical techniques and propose that these cells contain a bioactive peptide heretofore not identified in mammals. Our studies also indicate that these cells are nonproliferative and may arise by differentiation of basal cells. Hamsters treated with the carcinogen diethylnitrosamine revealed an increase in size and number of lung neuroepithelial bodies (NEBs), a second category of lung neuroendocrine-like cells. These cells could be dissociated from the lungs of treated animals and survive in short-term culture with retention of histochemical and ultrastructural characteristics of component cells of NEBs. The cultured cells could also be stained for ACTH-like immunoreactivity, a property previously observed with human bronchial carcinoids. All extracts of carcinogen-induced rodent squamous cell carcinomas and clonal subpopulations contained comparably low levels of ACTH by radioimmunoassay. Evidence of large molecular weight forms of the hormone and increased levels of immunoreactivity following cell culture suggests that de novo synthesis of the hormone occurs in these tumors.

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 argyropholic cells. Most of the antisera to various polypeptide hormones was 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 below. Other methods used were autoradiography, radioimmunoassay, and gel filtration column chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: The present program consists of investigations of neuroendocrine-like cells of the pulmonary epithelium, proliferative responses of these cells induced by carcinogens, and the occurrence of corticotropin-like immunoreactivity in various carcinogen-induced rodent squamous cell carcinomas. Essentially three major investigations, as described in last year's report, have been completed and are presently being prepared for publication.

(1) Neuroendocrine-like (K) cells of the normal pulmonary epithelium. Antisera to nearly fifty known polypeptide hormones or neuropeptides were used in an attempt to identify potential bioactive substances in solitary neuroendocrine-like cells of the guinea pig tracheal epithelium. These cells were identified by argyrophilia and formaldehyde-induced fluorescence, as has been described for other epithelial neuroendocrine-like cells. With one notable exception, all the antisera failed to reveal immunopositive cells. Occasional epithelial cells contained a peptide that cross-reacts with an antiserum to the amphibian tachykinin physalaemin. Preliminary findings also indicate that some nerve fibers stained with antiserum to somatostatin. A previous report indicated that substance P may be present in cells of the guinea pig tracheal epithelium. We were not able to confirm this finding using two different antisera to this neuropeptide. We were able to reproduce our earlier findings that argyrophilic beaded nerve fibers are occasionally observed in the submucosa of the tracheal epithelium and near the basal region of the argyrophilic solitary cells.

Guinea pigs injected with ^{3}H-thymidine fail to reveal labeling of neuroendocrine cell nuclei after one hour. This suggests, as was proposed for neuroendocrine cells of the gastrointestinal tract that, these cells are not proliferative and may form by differentiation of epithelial basal cells.

Ultrastructural analyses of these cells are still in progress and when completed will show whether there is more than one class of solitary neuroendocrine-like cells in the lung of mammals.

(2) Proliferation of endocrine-like cells in lungs of hamsters treated with diethylnitrosamine (DEN). We demonstrated that hamsters treated with the systemic carcinogen DEN have hyperplastic neuroepithelial bodies (NEBs) after five weeks of treatment (3 mg s.c., twice weekly). The number of argyrophilic cells observed in lung sections of treated animals increased nearly 600% over controls. The number of NEBs increased at least threefold. Since exposure of these cells or their progenitors to a carcinogen may effect initiation and survival of the cells in tissue culture conditions, lung cells were dissociated with pronase and cultured. The dissociated cells were maintained in Ham's F12 medium with 10% fetal calf serum and the medium changed after 24 hr, and then finally replaced with L-15 medium (GIBCO) after 48 hr. After 7 days in culture the attached cells were fixed for morphological examination. The cultured cells initially obtained from hamsters treated for 5 weeks revealed argyrophilic cells (40%), cells with dense-cored vesicles (30%) and cells with ACTH-like immunoreactivity (16%). In cultures established with lung digests obtained from hamsters treated with DEN for 8 weeks, the number of cells with these properties in the 7-day cultures were apparent but considerably reduced in number, especially for cells with ACTH-like immunoreactivity. The correlation between the appearance of cells with ACTH-like immunoreactivity in culture and the hyperplasia of endocrine like cells induced by DEN in vivo suggests a relationship between these cells. The present study suggests that lung endocrine-like cells or their progenitors may be particularly sensitive to the carcinogenic effects of DEN.

Further studies are in progress to confirm that endocrine-like cells observed in vitro were derived from hyperplastic NEBs. A more thorough investigation will be made of the optimal culture conditions for survival or growth of the endocrine-like cells.

When this is achieved, we shall attempt to obtain cell lines and clonal subpopulations for the purpose of preparing specific membrane antisera and establishing other specific chemical markers as tools for understanding the origin of these cells in the lung epithelium. These studies should be helpful towards identifying early neoplastic effects associated with lung neuroepithelial bodies and native bioactive substances present in these structures.

(3) Occurrence of ACTH-like immunoreactivity in chronically-induced squamous cell carcinomas and cloned subpopulations. We had previously observed that extracts of transplantable rodent squamous cell carcinomas induced by localized exposure of tracheal transplants to benzo(a)pyrene or by intratracheal injection of 3-methylcholanthrene contain immunoreactive corticotropin (ACTH). The amount of ACTH-IR in these tumors ranged between 5 and 20 ng (gm/wet weight), which is similar to the amounts previously reported for corresponding human tumors. Because of the significance previously given to the ectopic formation of polypeptide hormones in detection of neoplasia, we analyzed various clonal subpopulations of rat and hamster lung tumors for ACTH-IR. Comparison of various clones and the parent line revealed no significant differences in immunoreactivity in extracts.

One clonal subpopulation originating from a hamster lung tumor was examined for net synthesis of ACTH-IR in vitro. It was very evident from this experiment that these cells in vitro synthesize an ACTH-like peptide and that the rate of synthesis was dependent on the growth rate of the cells. After plating, a rapid burst of specific content of hormone was observed (ACTH-IR/mg dry weight cells). As the cells in culture became confluent, the rate of synthesis decreased significantly.

Gel filtration column chromatography of extracts of two of the cell lines from chemically-induced lung squamous cell carcinomas revealed immunoreactivity at 35-40K and 3-5K. About 90% of the immunoreactivity was associated with the smaller peak, which may represent intact ACTH or a fragment of this peptide. The high molecular weight material may represent the known precursor form of the hormone.

These studies indicate that (1) carcinogen-induced lung squamous carcinomas resemble human lung tumors of the same histologic type because they contain immunoreactive ACTH at comparable levels, (2) the hormone in the tumors appears to be synthesized de novo, (3) the low-level synthesis of the hormone in the tumors discussed here, is a common property of all cells within the tumor and not a trait of a local subpopulation within the tumor. It is possible that expression of ACTH may be a general concomitant of neoplasia.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The usefulness of the measurement of specific proteins associated with neoplastic events has been previously underscored by many investigators in the field. In the present studies, we have shown that ACTH-immunoreactivity may be of significant value in the detection of early neoplastic events occurring in lung cells exposed to a carcinogen. This was particularly evident in studies where lung endocrine-like cells obtained from hamsters treated with diethylnitrosamine revealed marked ACTH-like immunoreactivity in vitro. These studies suggest the significance of neuroendocrine-cells of the lung or their progenitors as targets for systemic carcinogens of the N-nitrosamine class.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80035-04 LPFT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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
Other:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	J. Boyd	Temp. Technician	LPFT	NIEHS
	R. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

K. Sivarajah and J. Lasker, Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.95	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)

It is the long-range goal of this project to study the co-oxygenation of chemicals, i.e., benzo(a)pyrene (BP) 7,8-diol during the metabolism of arachidonic acid (AA) by guinea pig lung, ram seminal vesticle, mouse skin, human lung, and cells grown in tissue culture BP-7,8-diol was oxidized to 7,10/8,9 tetral prostaglandin synthetase by all the tissues examined. Electrophilic metabolites of BP7,8diol was covalently bound to protein. With human lung the rate of conversion of BP-7,8-diol by the AA dependent system was greater than the NADPH-dependent oxidation. AA-dependent oxidation produced only 7,10/8,9 tetral but NADPH-dependent oxidation formed 4 tetrols. We found that during the oxidation of AA to PG's, a number of aromatic amines (including several insecticides) are demethylated. The significance of the oxidation of chemicals during PG formation, as related to chemical-induced toxicity or carcinogenesis is being examined.

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues, such as guinea pig lung and ram seminal vesicles, were used to examine the co-oxygenation 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 AND PROPOSED COURSE: The environmental carcinogen, BP-7,8-diol, is oxidized during the conversion of arachidonic acid to PG's and TX. Lung, skin, gut, kidney, and seminal vesicle microsomes convert BP-7,8-diol to metabolites which migrated as a single peak on thin layer chromatography and corresponded to authentic samples of BP tetrols. The metabolites formed from BP-7,8-diol by the AA system, when examined by the HPLC, appeared to be 7,10/8,9 tetrols (major metabolite) with small amounts of 7,8,9/10 tetrol. The cytochrome P-450-dependent oxidation of BP-7,8-diol could generate 4 tetrols that come from diol epoxide I and II (two tetrols from each). It appears that the two tetrols generated by the AA system come from diol epoxide I. The diol epoxides are the ultimate carcinogens of BP. Of the two diol epoxides, diol epoxide I is supposedly more carcinogenic. A recent report indicated that the AA-dependent metabolism of BP7-8, diol generated metabolites highly mutagenic towards Salmonella typhimurium. The metabolites generated by the AA system from BP-7,8 diol BP by lung and ram seminal vesicle microsomes were the same in our HPLC analysis. BP-7,8-diol was oxidized by the AA-dependent system to metabolites which were electrophilic as indicated by BP-7,8-diol associated radioactivity remaining covalently linked to protein.

AA-dependent oxidation of BP-7,8-diol by human pulmonary tissues was compared to NADPH-dependent oxidation using human lung tissue. In all cases the rate of AA-dependent oxidation of BP-7,10/8,9 tetrol but NADPH-dependent oxidation produced all four tetrols with the 7,10/8,9 tetrol being the major metabolite. We found that AA-dependent oxidation can occur at very low enzyme levels.

We have also examined the oxidation of BP-7,8-diol in tissue culture (10T 1/2 cells). On addition of AA to cells, there is a stimulation of PG synthesis. This results in a stimulation of BP-7,8-diol oxidation. We intend to characterize the system, measure binding to DNA and study cell transformation.

A number of aromatic amines can also be oxidatively demethylated during the formation of PG's from AA-PCMA, dimethylaniline, the insecticides amino-carb, and amino-pyrine was oxidized by the AA-dependent system at greater rates than the NADPH cytochrome P-450 system. The oxidation of amines was dependent on the generation of the hydroperoxide PGG_2 from AA. The K_m values for

oxidation by AA-dependent and the P-450 systems were similar. The stoichiometry for AA-dependent oxidation varied from 9 to 120 (oxidation/hydroperoxide). The reaction was inhibited by indomethacin, phenylbutazone and BHA but not by SKF-525-A, or metyone.

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 test 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.

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 106:17-2, 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 (Submitted)

Sivarajah, K., Lasker, J., Abou-Donia, M., and Eling, T. E.: Metabolism of N-alkyl compounds during the biosynthesis of prostaglandin. J. Biochem. (Submitted).

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, 1979 - September 30, 1980

CURRENT ANNUAL LEVEL: $165,000

PROJECT DESCRIPTION

OBJECTIVES: Our research work was directed toward:

1) The morphological characterization of dark epithelial cells in preneoplastic lesions produced by the chemical carcinogen, dimethylbenzanthracene (DMBA).

2) Comparing the dark cells of DMBA induced lesions with similar cells found in squamous metaplasias produced by non-carcinogenic agents in the rat trachea.

3) Investigating the distribution and characteristics of dark cells in human "spontaneous" preneoplastic lesions of the airways.

4) Establishing of a two-stage carcinogenesis model in the tracheal transplant.

5) Repopulating denuded tracheas with several initiated cell lines in order to establish the characteristics of the repopulated epithelia and their evolution.

METHODS EMPLOYED: Dark epithelial cells were studied in Epon sections stained with toluidine blue. The material was originated from heterotopic rat (Fischer 344) tracheal transplants exposed to 165-200 µg DMBA for 4 weeks. The lesions were harvested 16 to 48 weeks after cessation of DMBA exposure. Squamous metaplasias of the trachea were obtained from Fischer rats fed for four to five months with vitamin A deficient diet or from rats treated during 6 months with topical administration of formaldehyde 40% once a week. All animals were injected with tritiated thymidine prior to sacrifice, enabling us to perform autoradiograms from the Epon embedded tissues. The human lesions were obtained from 21 autopsy cases from the Department of Pathology, St. Mary's Hospital, Grand Junction, CO (Dr. G. Saccomanno), reembedded in Epon, and the sections stained with toluidine blue.

The two stage carcinogenesis experiments (initiation with DMBA and promotion with TPA) were performed using rat heterotopic tracheal transplants, and the lesion analyzed in routine paraffin sections stained with HE.

The repopulation studies were carried out injecting several DMBA initiated cell-lines into denuded rat tracheas (the original epithelial layer is destroyed by repetitive freezing and thawing) which were then trnasplanted into nude mice of Balb background. The cell-line-originated epithelia which grow in the tracheal transplants were studied using Epon sections 2, 4, 12, and 24 weeks after inoculation of the cells.

MAJOR FINDINGS AND PROPOSED COURSE: Dark basal cells were observed in both human and rat preneoplastic lesions of the respiratory tract epithelium. The percentage of dark cells in the basal and suprabasal layers increased in direct proportion to the degree of atypia. In the rat, DMBA induced squamous metaplasias without atypia showed 18% dark cells in the basal layer and 0.2% in the suprabasal layers; the squamous metaplasia with moderate atypia exhibited 43% and 3% in the basal and suprabasal layers, respectively; and the squamous metaplasia with severe dysplasia showed 57% in the basal and 28% in the suprabasal layers. In carcinoma in situ, the percentage of dark cells reached a maximum of 71% in the basal and 56% in the suprabasal layers. In the human lesions, the tendency was the same but the percentages of dark cells in the basal layer were not as high as in the experimental rat lesions, e.g. squamous metaplasia without atypia 9%, the percentages increased gradually up to 34% in carcinoma in situ.

Dark cells were also found in squamous metaplasias produced in the rat by noncarcinogenic agents. Formaldehyde induced squamous metaplasias exhibited 4% dark cells, whereas vitamin A deficient animals showed 18% basal dark cells in similar lesions. The labeling index of basal cells in metaplastic epithelia, regardless of the inducing agent was 17-18%, i.e. the same as that of the normal esophageal stratified squamous epithelium. The percentage of labeled dark basal cells per total basal cell population was less than 5% in the non-carcinogen-induced metaplasias and in the DMBA-induced metaplasias without atypia. In the atypical metaplasias induced by DMBA, this percentage increased to 13. On the basis of ultrastructural observations, five types of dark epithelial cells could be distinguished in the metaplastic epithelia: Type I (ovoid or fusiform dark cell with abundant cytoplasmic filaments, desmosomes, and free ribosomes = basal keratinocyte type), Type II (ovoid or spherical small cell with scant cytoplasm with few organelles = basal respiratory type), Type III (irregular or ovoid, few cytoplasmic filaments and organelles and desmosomes, extremely abundant free ribosomes = dedifferentiated type), Type IV (fusiform or ovoid, large mitochondria, prominent ergastoplasm, secretion droplets = mucus-cell type), and Type V (irregular shape, organelle remnants, vacuoles, pycnotic nuclei = involutional-cell type). Type I was the predominant cell type in formaldehyde-induced metaplasias and was also commonly seen in DMBA-induced metaplasias without atypia. Type II predominated in vitamin A deficiency-induced metaplasias. Type III was seen in DMBA induced metaplasias and was the predominant cell type in the atypical epithelial alterations. Type IV cells occurred only in the latter and Type V cells were occasionally seen in formaldehyde as well as in DMBA-induced atypical metaplasias. Each type of squamous metaplasia could thus be recognized by a determined numerical distribution of dark cells in the basal layer and a specific pattern of distribution of the ultrastructurally defined dark cell categories.

Several experimental groups for the study of two stage carcinogenesis in tracheal transplants, using DMBA as initiator and TPA as promoter, have been already set up. Unfortunately, most of these groups will have to be repeated because of the nature of the vehicle used. The vehicles for the initiator and promoter pellets contain either 90% or 20% cholesterol in beeswax (which permits a slow release of the agents). It was found that the blank pellets containing neither DMBA nor TPA (but containing 90% cholesterol) are able to induce the formation of stratified and squamous metaplasias in the tracheal epithelium. The most probable cause of these phenomena is the presence of cholesterol oxidation products, which recently have been shown to be mutagenic. We are now in the process of utilizing oxidation products-free cholesterol, which hopefully will permit the continuation of our two stage carcinogenesis experiments.

Twenty cell-lines originally derived from tracheal epithelium treated with subtumorigenic or low tumorigenic doses, have been inoculated in denuded rat tracheas, which were transplanted s.c. into nude mice. Four of these cell-lines produced squamous cell carcinomas before 20 weeks, 3 grew initially covering the lumina but were no longer found after 12 weeks (the lumina were occluded by granulation tissue), and the rest covered the tracheal walls with stratified squamous or transitional epithelium (3), with cuboidal epithelium containing patches of mucociliary cells (3), or a mixture of all these types (7). After completion of this study, cell lines with different morphology and/or different LI will be exposed to TPA and to low doses of DMBA in order to investigate the influence of initiating and promoting agents on already initiated epithelial cells which exhibit different differentiation and morphology.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The purpose of the studies is to develop a better understanding of the morphological and biological characteristics of putative preneoplastic lesions in respiratory tract epithelium. They will provide information on the environmental and host factors which govern the progression of preneoplastic changes to invasive carcinomas. This knowledge is essential to design, in the future, rational approaches for intervention therapy in groups of individuals at high risk to develop cancer. This work will also provide data which will help to decide whether preneoplastic lesions can be used as endpoints in short term *in vivo* carcinogenesis bioassays.

PUBLICATIONS

A. J. P. Klein-Szanto, D. C. Topping, C. A. Heckman, and Paul Nettesheim. Ultrastructural characteristics of carcinogen induced non-dysplastic changes in tracheal epithelium. Am. J. Pathol. 98:61-62, 1980.

A. J. P. Klein-Szanto, D. C. Topping, C. A. Heckman, and P. Nettesheim. Ultrastructural characteristics of carcinogen induced non-dysplastic changes in tracheal epithelium. Am. J. Pathol. 98:83-100, 1980.

A. J. P. Klein-Szanto, J. N. Clark, and D. H. Martin. Sexual differences in the distribution of epithelial alterations in vitamin-A deficient rats. Int. J. Vitam. Nutr. Res. (in press).

A. Klein-Szanto, D. H. Martin, and A. H. Pine. Cutaneous manifestations in rats with advanced vitamin-A deficiency. J. Cutan. Pathol. (in press).

P. Nettesheim, A. J. P. Klein-Szanto, A. C. Marchok, V. E. Steele, M. Terzaghi, and D. C. Topping. Studies of neoplastic development in respiratory tract epithelium. Archiv. Pathol. (in press).

A. J. P. Klein-Szanto, A. Olson, and P. Nettesheim. Quantification of histological parameters in epithelial preneoplastic lesions of the trachea. Lab. Invest. 42:129, 1980 (abstract).

A. J. P. Klein-Szanto and P. Nettesheim. Different distribution frequency and ultrastructure of dark cells in carcinogen and non-carcinogen induced squamous metaplasias of the trachea. Proc. Am. Assoc. Cancer Res. (in press) 1980. (abstract).

UNIVERSITY OF CALIFORNIA
LAWRENCE LIVERMORE NATIONAL LABORATORY
Livermore, California 94550
(222Y01-ES-80040)

TITLE: Cytochemical Markers of Neoplastic Development in Tracheal Epithelium

CONTRACTOR'S PROJECT DIRECTOR: Martin Vanderlaan, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $60,000

PROJECT DESCRIPTION

OBJECTIVES: Dr. Vernon Steele at NIEHS is working on developing an *in vitro* transformation model for rat tracheal epithelium. This project is run in collaboration with his, and seeks to identify morphological and biochemical markers with which early transformed rat tracheal cells may be recognized. Cells are being put into culture at NIEHS and exposed to various whole carcinogens and promoters. Treated cultures are shipped to Livermore where the cells are analyzed for DNA content, nuclear size and shape, cell size, and nuclear/cytoplasmic ratio. When these initial markers have been evaluated, cytoenzymologic and immunologic methods will be used to stain for potential enzyme and antigenic markers of the cells.

METHODS EMPLOYED: The project is set up as an iterative one, with close feedback between the development of the transformation assay at NIEHS and the recognition of markers for transformed cells at Livermore. "Small-cell foci," which are the earliest morphological feature readily recognized in carcinogen-exposed cultures, are compared with other cells on the culture dish. Comparisons are made "*in situ*," i.e., on cells as they grow on the plastic culture dishes. In this way each cell retains its position vis-à-vis its neighbors and morphologic comparisons can be made between cells which are close to one another (and therefore likely to have a recent common parental cell) and those that are more distant. The acute toxic effects of carcinogens on cell morphology are also being determined.

Morphologic analysis is being made using a fluorescence scanning microscope (FLEX) developed by Dr. I. T. Young of the Biomedical Division, Livermore. The complete FLEX system is designed to acquire high resolution fluorescence images from cells on slides or culture dishes, digitize the images, and store the images in a central computer. Software programs either exist or are being written which permit morphometric analysis of the stored images, including determination of nuclear size, shape, total nuclear fluorescence intensity (DNA content), cell size, and total fluorescence intensity of the cell.

MAJOR FINDINGS AND PROPOSED COURSE: To date most effort has been placed on developing the hardware and programming of the FLEX system, standardizing the image acquisition procedure, and validating the cytochemical staining methods. For these latter studies, cultured cell lines of tracheal cells have been used. Propidium iodide is being evaluated as a nucleic acid stain and fluorescamine provides a counterstain for total cell protein. Propidium iodide stains the nuclei red, with some red in the cytoplasm resulting from cytoplasmic RNA and non-specific binding. Fluorescamine reacts with amino groups of proteins, giving a blue fluorescence. Factors influencing staining such as stain concentration, staining time duration, pretreatment with RNAse, fixation of the cells, and stability during storage of the stained cells, are being evaluated.

All cytochemical staining procedures require that the FLEX system provide valid quantitative data on the cells. As such, much of our efforts have been directed at the FLEX instrumentation. For example, the fluorescence image is acquired first by a SIT vidicon TV camera. Software programs have had to be written that compensate for any spatial non-uniformities in the sensitivity of the camera, any degree of non-linearity in the camera response, and setting the threshold and gain for camera response. All of this programming has now been completed, and the FLEX system as a whole is about ready to be turned over completely to users. Dr. Vanderlaan is at present the main user of the system, and has been actively involved in its design, validation, and in developing the cytochemical staining methods for cytologic samples.

During the course of these studies at Livermore, the in vitro transformation assay has undergone some major revisions in methodology. In particular, primary cultures of epithelial cells are now being grown on collagen-coated dishes. This has made a significant increase in the plating efficiency, and means that a whole new cell population is being studied. Thus, the specific cytochemical measurements made previously on primary cultures may have little significance for the new transformation protocol.

Specific results on cultured cells to date are sparse for the above reasons. We believe that the collaborative efforts between Livermore and NIEHS are now in a position where very fruitful measurements can be made in the near future, however. The recent improvements in instrumentation, cytochemistry, and the culture of tracheal cells will mean that the next 6-12 months can be devoted specifically to the development of a data base on cultured tracheal cell morphology during the course of neoplastic transformation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In vitro assays for transformation of epithelial cells, particularly tracheal epithelial cells, are very important if we are to gain an understanding of the basic biology of lung cancer cells. Such a model system will provide material for the study of carcinogenesis, and may lead to a short-term assay system for testing carcinogens. One problem with the transformation of epithelial cells is that it is often difficult to recognize the transformed state in cells in culture. In general, the transformed phenotype is not like that of transformed fibroblasts, and one cannot use the loss of contact inhibition or the tendency

to form piled-up foci as a means of recognizing transformed cells. As such, new methods based on morphology or biochemistry of the cells will have to be developed. Quantitative image analysis of cells using scanning microscopy offers the potential of recognizing those cells which have undergone one or more of the steps in neoplastic progression.

PUBLICATIONS

No publications have resulted so far from this study at Livermore, other than the programs for FLEX and an in house users guide for FLEX.

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. During the last year, we have confirmed that these cell lines progress from non-tumorigenic to tumorigenic cell populations during passage in vitro and have frozen banks of cells at various stages of this progression. The development of transplantation-resistance in syngeneic animals and the identification of specific cellular and humoral immune responses have established that these tumors are immunogenic in syngeneic animals. Anti-tumor antibody was detected in sera of immune animals by a radiolabeled antibody binding test and by indirect fluorescence testing. Antisera derived from tumor-immune animals have been characterized for their specificity of reaction and titer. Cell-mediated immune responses of spleen cells from tumor-immune animals was demonstrated by appropriate tumor target cell killing. Antigenic determinants recognized by tumor immune lymphocytes were limited to neoplastic tumor cell lines and did not appear to be present on the normal, non-carcinogen treated primary cultures. Assays showed that both humoral and cell-mediated responses were directed against antigenic determinants on the surface of neoplastic tracheal cell lines, and were not present on normal, non-transformed primary epithelial cultures. These reagents are currently being used to identify the appearance of these tumor markers after carcinogenic exposure of normal tracheal explants and to quantitate the expression of antigen as the cell progresses from the normal to neoplastic cell.

METHODS EMPLOYED: Induction of transplantation immunity (the ability of an animal to resist tumorigenic inoculum) has been used to determine if tumors are immunogenic. Animals that demonstrate transplantation resistance are being used as a source of immunologic reagents to identify and quantitate neoplastic tumor antigens. Serum from tumor-immune animals was assayed for antibody activity by the antibody binding test (ABT) developed in our laboratory. Briefly, cells to be used as targets were plated on glass coverslips, and when confluent overlayed in the cold with diluted test serum. Binding of the rat antibody to the target cell monolayer was quantitated by using ^{125}I-labeled

antiserum for determining the presence of these markers on individual cells. Specificity of tumor-immune antisera was determined by qualitative absorption before testing as well as by direct binding of unabsorbed antiserum on other cell targets in the ABT. Two methods are being used to identify cell-mediated immune responses. Spleen cell populations from both tumor-bearing or tumor-immune animals are being tested for (1) cytotoxicity on various tumor target cells; and by (2) blastogenesis of immune lymphocytes by x-irradiated tumor cells (stimulation of lymphocyte DNA synthesis in 6 day in vitro cultures) as determined by tumor-lymphocyte interactions (TLR).

MAJOR FINDINGS AND PROPOSED COURSE: Two tumorigenic (late passaged) cell lines induced by MNNG have been used to immunize several syngeneic animals. Serum from individual rats have been tested for antibody activity by the ABT on autologous tumor target monolayers. In all cases, antibody was detected in serum of immunized rats; however, the reactivity of tumor-immune serum for autologous tumor targets showed considerable variation. Since all of the monolayers were at similar passage levels, these differences are considered to be due to differences in relative antibody titers in sera of individual aniamls. Antiserum determined positive by the ABT also showed positive fluorescence staining on homologous tumor target monolayers. Normal rat serum with no demonstrable antibody activity for tumor cells showed no fluorescence when tested on tumor target monolayers at similar dilutions. To determine if antigens recognized by the anti-tumor serum appeared on a subpopulation of normal non-carcinogen treated epithelial outgrowths, tracheal explants were plated on coverslips and overlayed with immune rat sera. There was no evidence of fluorescence staining on these normal epithelial outgrowths, while tumorigenic cultures were strongly positive. Thus, determinants recognized by tumor immune serum do not appear to be present on the non-transformed, epithelial cell outgrowths. These studies are continuing on both normal and preneoplastic cell cultures.

Spleen cells from tumor-immune or tumor-bearing rats were also tested for cytolytic activity (CTL) either directly (1°-CTL) or after 6-day in vitro co-cultivation with lethally irradiated tumor cell cultures (2°-CTL). These results can be summarized as follows: (1) Cytotoxicity occurred only when the sensitizing population bears appropriate tumor marker on its cell surface. It was clearly documented that these antigens did not appear on normal epithelial cultures by this method. (2) Spleen cells originating from tumor-immune animals showed considerable cross-reactivity between the MNNG and DMBA induced tumorigenic cell lines. (3) Although secondary CTL also showed cross-reactivity, preliminary evidence indicates that the cytotoxic response becomes more specific for the immunizing tumor after in vitro exposure to lethally irradiated tumor cells.

Having characterized the immunological response of syngeneic animals to tumorigenic MNNG induced tracheal carcinoma cell lines, we are now prepared to identify individual antigenic specificities in tumor-immune antiserum. The first experiments are designed to define whether there are determinants on late passaged neoplastic cell lines not present on preneoplastic cells of the same cell lineage, as well as antigenic determinants shared by both neoplastic and preneoplastic cultures. Using methods already developed, we

can also characterize when after carcinogen exposure these tumor antigens appear and if the developing preneoplastic cell lines are heterogeneous or homogeneous for presentation of specific antigenic determinants.

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 the exposed population before the appearance of neoplastic cell populations. This will enable us to determine if antigen positive cells in the exposed population before the appearance of neoplastic cell populations. 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 changes as a function of *in vitro* neoplastic differentiation. Assay systems used in these experimental animal model systems in the future will provide the basis for defining human respiratory tract tumor markers.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY
Summary Statement

Reproductive and developmental toxicology has generally lagged behind fields such as cellular and molecular biology with regard to laboratory approaches. The detection of environmental agents which affect reproduction or product birth defects is unsure and the underlying biological mechanisms which account for these major health problems are unclear. Thus, the Laboratory of Reproductive and Developmental Toxicology bridges 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. Studies are conducted focusing on the entire biological spectrum from molecular aspects to whole animals. Organizationally, the Laboratory consists of four administrative units or workgroups: Molecular Embryology, Experimental Teratogenesis, Transplacental Toxicology, and Germ Cell Toxicity. However, extensive collaboration exists between these groups; thus, the current research accomplishments 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 cloning for androgen dependent rat prostate genes.
- o Molecular characterization of androgen dependent rat seminal vesicle genes.
- o Protein-mapping of the developing genital tract of the fetal and neonatal mouse.

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.

II. Ultrastructural/Cellular Level

A. Ultrastructural Changes as Predictors of Functional Abnormalities

- o Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histologic 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 teratology.
- 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 rat fetuses during critical periods of organogenesis.

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 Morphologic 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.

IV. Whole Animal Studies

A. Toxicology

- o The teratogenicity of anticonvulsant agents and structurally related chemicals.

- 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 Quantitation 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.

Summaries of these projects are presented below; details of the work appear in the individual annual reports.

I. Molecular Level

A. Hormone-Related Gene Action in the Male and Female Genital Tract

Hormone regulation of prostate and seminal vesicles protein synthesis. Protein synthesis patterns analyzed by two dimensional (2D) gel electrophoresis in the prostate and seminal vesicle of castrate 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 γ) 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 codes for a larger (22,000 dalton) secretory protein referred to as alpha (α). Likewise, rat seminal vesicle has two major poly(A^+)-mRNA's (40%) which code for two major seminal vesicle proteins which are androgen responsive (IV and V).

Molecular cloning for androgen dependent rat prostate genes. Double-stranded complementary DNA (ds cDNA 10-13S) to androgen dependent prostate β, γ, and α poly(A^+)-RNA was prepared using reverse transcriptase. The synthetic gene ds cDNA β was separated from the others by preparative agarose gel electrophoresis. The relatively pure ds cDNA to β was then restricted with a variety of restriction enzymes and a preliminary map generated for this synthetic prostate gene. A large batch of ds cDNA 10-13S was synthesized, treated with S1 nuclease, tailed with terminal deoxynucleotide transferase, and cloned in the plasmid pBR322. Clones were identified which had a high probability of containing β ds cDNA inserts by colony filter hybridization. Other clones containing inserts coding for polypeptides α and γ, as well as the β clones, were identified by hybrid arrest translation assays. Restriction maps for the clones containing coding sequence for α and β polypeptides have been developed.

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, 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 $\cong$ 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.

Protein-mapping of the developing genital tract of the fetal and neonatal mouse. The protein-mapping technique of O'Farrell is being used to study the synthesis of total protein in fetal and postnatal mouse genital tracts. Normal differentiation in Müllerian and Wolffian ducts from mouse fetuses beginning with day 14 through birth is being followed by ^{35}S-methionine incorporation

into protein. High resolution maps of immature and mature mouse uteri are also being developed to establish end points of differentiation. There are approximately 400 protein spots detectable in each pattern developed thus far. Some 5% of the detectable proteins are either sex or developmental stage specific. Prenatal diethylstilbestrol exposure results in an abnormal fetal uterine protein synthetic pattern which persists into sexual maturity suggesting induction of altered cell differentiation.

B. Biochemical Basis of Estrogen Action

Characterization of nuclear events involved in estrogen action in the mouse uterus. Characterization of uterine nuclear estrogen receptors in the mouse suggests two forms of the receptor, since 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. 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 resynthesis, RNA polymerase activities, DNA polymerase activities and glucose oxidation/-utilization are also being investigated. Recent studies using steroid autoradiography raise the possibility that the second nuclear accumulation of estragiol-receptor complex may be related to its redistribution to different cell types within the tissue. Thus, estrogen action in the mouse uterus may involve sequential stimulation of various tissue compartments for its expression.

Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites. In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo 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 and possible metabolites showing receptor binding comparable to DES, but 20-100 times less biological activity. These studies have suggested a mechanism of altered/differential clearance of these 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.

II. Ultrastructural/Cellular Level

A. Ultrastructural Changes as Predictors of Functional Abnormalities

Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histologic 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 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 IPT 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 teratology. 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 ellicit a biological response include 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 analyses 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 the 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 epidemiologic 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. LRDT's 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 morphologic abnormalities of the offspring.

III. Tissue/Organ Level

A. Cultured Embryos

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain whole rat fetuses during critical periods of organogenesis. To aid in the laboratory assessment of teratogens and the understanding of the molecular mechanisms underlying teratogenesis, an in vitro culture system for rat 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.

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 morphologic 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.

Morphologic 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 Müllerian Inhibiting Hormone.

Morphological characterization of long-term fetal tissue grafts. To overcome difficulties in long-term (greater than two months) maintenance of fetal tissues in vitro, cultured fetal gonads or genital tracts are carried as grafts in appropriately manipulated hosts. Under these conditions, fetal ovaries develop into functional gonads when grafted in 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 nor 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.

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, ethotoin 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 share a common moiety and all are embryotoxic and teratogenic.

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. 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 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, and suggest the need to examine in human males exposed to Kepone and to assess potential deficiencies in sperm fertilizing capacity using the heterologous in vitro 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.

Quantitation 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 simply dose is established. The relationhsip 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 capcity 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 increase the reliability of extrapolating laboratory animal data to man and estimating human risk.

NIH CONTRACTS

Dr. McLachlan and Dr. Lucier of the Laboratory of Organ Function and Toxicology are project officers for a contract with the Wisconsin Alumni and Research Foundation which studies the transplacental toxicity of selected environmental chemicals.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international level. 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. Dixon has continued a productive collaboration with Dr. Sergio Fabro of the George Washington University. Dr. Fabro has been advisor to the Experimental Teratogenesis Workgroup which is concerned with mechanisms of teratogenesis.

Dr. Hall has continued 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. Harris is involved in a collaborative project with Bethesda Research Laboratories concerning sequencing the DNA coding for rat seminal vesicle protein IV, a major androgen dependent gene product of the rat seminal vesicle.

Dr. Lee is involved 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 prenatally exposed 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 involvement.

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 is also involved in collaborative DES studies with the Institute for Pharmacology and Toxicology, University of Wurzburg, Germany.

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 health research. Descriptions of representative examples for the Laboratory are given below:

Dr. Dixon has a large number of committee and program assignments which augment and are relevant to the NIEHS mission such as Chairman of the Committee on Environmental Pharmacology of ASPET, Committee on Women in the Workplace organized by NIOSH, Project Director of the teratogenic and reproductive effects areas of the NIEHS Energy-Related Research Program, 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. His appointment as Assistant to the Director for International Programs involves Dr. Dixon in the NIEHS' international activities including cooperative agreements with foreign countries, ad hoc international activities in response to particular scientific problems, temporary employment of foreign scientists, and informal exchanges of scientists (and technicians). This assignment encompasses current agreements between the US and the Soviet Union, Japan, Egypt, Italy, Peoples' Republic of China, and the World Health Organization (WHO) as well as future cooperative activities which will be developed. In addition, Dr. Dixon has been co-organizer of several Target Organ Toxicity Symposia in cooperation with the Society of Toxicology. Proceedings of this symposia are published in Environmental Health Perspectives and include symposia on liver, kidney, lung, goands, development, nervous system, cardiovascular system, intestines, blood, and endocrine system.

Dr. McLachlan is a member of the DHHS Taks Force on DES toxicity and has been advisor to NIOSH and the FDA on the toxicity of estogens.

Dr. McLachlan organized a symposium on "Estrogens in the Environment" sponsored by the NIEHS. Dr. Korach served as a member of the organizing committee for the symposium.

Dr. McLachlan and Dr. Korach co-organized a Target Organ Toxicity symposium on the Endocrine System.

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 on the International Journal for Biological Research in Pregnancy.

TRAINING PROGRAMS

Environmental helath 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, Dixon, Korach, and Hall.

Drs. Hall, McLachlan, and Korach have lectured at UNC and/or Duke in areas of their research expertise. Dr. McLachlan has also lectured at the Bowman-Gray School of Medicine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70001-04 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Teratogenicity of Antiepileptic Drugs and Chemically Related Compounds

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

PI:	R. L. Dixon	Laboratory Chief	LRDT	NIEHS
OTHER:	S. Fabro	Visiting Scientist	LRDT	NIEHS
	J. Kao	Visiting Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

George Washington University Medical Center

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	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)

Clinical observations have strongly implicated a number of anticonvulsants as human teratogens. Compounds from 3 classes of anticonvulsants: oxazolidinediones, hydantoins and succinimides together with phenacemide and valproic acid have been evaluated, and found to be embryotoxic in a dose related manner in the CD-1 mouse. Following i.p. administration, these compounds produced not only intrauterine growth retardation, but also a range of congenital abnormalities. Defects of the skeletal system were primarily the result of treatment during early organogenesis while treatment during late organogenesis produced predominately cleft palates. Estimation of the relative teratogenic potentials of these compounds showed trimethadione, diphenylhydantoin, phenacemide and valproic acid to be potent teratogens in the mouse. Treatment of the dams on days 8-10 of pregnancy with valproic acid produced high incidences of exencephaly. Postimplantation mouse embryos, cultured in the presences of valproic acid produced defects in the neural tube. These defects were similar to those observed following treatment in vivo. This suggests a direct dysmorphogenic action of valproic acid on the developing central nervous system producing the condition described as exencephaly.

PROJECT DESCRIPTION

METHODS EMPLOYED: Lethalities were estimated in non-pregnant female CD-1 adult mice following three consecutive, daily i.p. administrations of the selected anticonvulsants. Lethality curves were obtained by analysis assuming an underlying probit model using the SAS probit analysis computer program. Dams were treated by single, i.p. injection of the test compounds on days 8, 9, and 10 or 11, 12, and 13 of pregnancy (plug date = day 1). The highest dose of the test chemicals used in embryotoxic and teratogenic evaluation was selected to be within the 95% confidence limits of the estimated adult LD_{01}. Methylcellulose solution (0.5% w/v) was the vehicle and appropriate controls were studied in parallel. On day 18 of pregnancy, the animals were sacrificed and laparotomy performed. Uteri were removed and the number of live, dead, and resorbing conceptuses recorded. Each live fetus was weighed and examined for external and internal abnormalities. Fetal heads were fixed in Bouin's solution and examined by Wilson's free hand slicing technique. Visceral examinations were performed by dissection with the aid of a stereomicroscope using the techniques outlined by Staples. Sketetal examinations were carried out after the decapitated and eviscerated fetuses were cleared and stained by the modified KOH-Alizarin Red S method.

The relative teratogenic potency of these selected anticonvulsants were estimated by obtaining a relationship between their teratogenicity following treatment on days 8-10 of pregnancy and their maternal lethality. Thus ratios of the computer estimated dose which produced 1% lethality (LD_1) and the dose which produced a 5% increase in malformation (TD_5) the relative teratogenic index (LD_1/TD_5) for each compound provides a measure of their relative teratogenicity.

Early organogenesis CD-1 mouse conceptuses were explanted on day 9 1/2 (plug day = 1) and dissected free of maternal decidua and Reichert's membrane. The embryos (3-5 somites) within the yolk sac and amnion were cultured for 24 hours or 48 hours at 37.5°C in male rat serum containing various concentrations of valproic acid. All operations were carried out aseptically and no antibiotics were used throughout the study. At the end of the culture, embryos and associated membranes were measured and examined. Growth was estimated by crown-rump and head measurements and DNA and protein determinations. Several morphological parameters were evaluated and the degree of embryonic differentiation was estimated using a morphological scoring system developed in this laboratory.

MAJOR FINDINGS AND PROPOSED COURSE: The antiepileptic drugs tested are structurally related and are derivatives of cyclic imides. Thus, trimethadione, dimethadione, and paramethadione are derivatives of oxazolidinedione; diphenylhydantoin, ethotoin and mesantoin are hydantoins; and phensuximide, methsuximide and ethosuximide belong to the succinimide class of compounds. However, phenacemide is an acetylurea, while valproic acid is a fatty acid

and does not share structural relationships with the other imide anticonvulsants. All the drugs and each of the basic chemical compounds produced embryotoxic and teratogenic effects. The malformation profile observed in the CD-1 mouse are similar to those described in humans following in utero exposure as in anticonvulsant therapy during pregnancy. Comparison of the relative teratogenic index of these compounds showed trimethadione, diphenylhydantoin, phenacemide and valproic acid to be significantly teratogenic in the mouse. The finding that they are teratogenic suggests the possibility that the dysmorphogenic potential of these compounds might be a property of their pharmacological activities.

The in vitro system supported growth and differentiation of cultured mouse embryos. At the end of the culture periods, embryos developed to stages which were morphologically indistinguishable from embryos maintained in vivo. However, embryos cultured for 24 or 48 hours in the presence of valproic acid resulted in a dose-related retardation in growth and differentiation. In addition, a dose-dependent variety of dysmorphogenic effects was observed in these embryos. Irregularity of somites was most evident. This was often associated with irregular fusion of the telencephalic and mesencephalic regions. These findings were similar to those observed following treatment in vivo. Additionally, stunted telencephalic hemispheres were observed.

These results suggest a direct teratogenic action of valproic acid. This action is often associated with retardation in growth and differentiation and produce dysmorphogenic effects on developing systems in vitro similar to those observed in vivo. This culture system, therefore, permits the study of the effect of teratogens not only with regard to the induction of dysmorphogenesis and retardation in growth and differentiation of postimplanted embryos, but also provides additional information to that obtained in conventional screening for the teratogenic potential of chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In humans, anticonvulsant therapy during pregnancy has been associated with increased incidences of birth defects. However, such conclusions are largely based upon retrospective analysis of records, and the etiology of congenital abnormalities is often confounded by the influence of epilepsy per se. This study shows that selected anticonvulsant drugs are teratogenic in the CD-1 mouse, producing a spectrum of malformations which includes many of the abnormalities seen in humans. Thus, the CD-1 mouse appears to be a good animal model for use in studies of the relative teratogenicity of these agents. The comparison of relative teratogenic potential of these antiepileptic drugs may provide useful information for estimating the human risk-benefit relationship of anticonvulsant therapy during pregnancy. In addition, the successful development of the in vitro culture technique may provide a system whereby processes associated with chemically induced dysmorphogenesis may be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70010-04 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study of Reproductive and 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. L. Dixon	Laboratory Chief	LRDT	NIEHS
OTHER:	S. Fabro	Consultant	LRDT	NIEHS
	B. P. Schmid	Visiting Fellow	LRDT	NIEHS
	J. Kao	Visiting Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

George Washington University Medical Center

LAB/BRANCH
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SECTION
Experimental Teratogenesis Group

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 t

The objectives of this project are: (1) To culture postimplantation embryos to aid the laboratory assessment of potential teratogens and the understanding of the molecular mechanisms underlying teratogenesis; (2) to utilize in vitro fertilization (or zygotes) coupled with preimplantation embryo culture, transfer of blastocysts to pseudopregnant recipients and evaluation of "pregnancy outcome" to identify critical early development targets of environmental chemicals.

(*Formerly, Study of Developmental Disorders During Organogenesis in a Model Embryo Culture)

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 10, 11 or 12, are cultured in heat inactivated homologous serum for 2-4 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.

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.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Pregnancy day 10 rat conceptuses, cultured for 43-45 hrs are highly sensitive to tetraethylene melamine (TEM) exposure. Embryonic development was abnormal at a dose level as low as 0.05 µg/ml medium. DNA and protein synthesis, differentiation of the neural tube, sensory organs, and somites were significantly affected by TEM.

(2) 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.

(3) 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.

(4) The effects of cadmium chloride on fertilization and early and late development were studied. Ova and sperms were exposed in vitro to three concentrations of cadmium chloride (0.1, 0.2 and 0.4 µg/ml) and their development compared to controls. The number of inseminated ova which cleaved,

formed morulae and blastocysts, implanted and developed into normal fetuses were recorded. Cadmium had no effect on fertilization nor 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 implanted, the percent of normal term offspring was unchanged by cadmium exposure.

The proposed course of this involves developing procedures for continuous in vitro growth and differentiation of embryos from preimplantation to organogenesis stages 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 processed during organogenesis will be evaluated. The early-late 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 reproduction and 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

Brown, N. A., Goulding, E. H., Fabro, S.: Ethanol embryotoxicity: Direct effects on mammalian embryos in vitro. Science 206: 573-575, 1979.

Brown, N. A., Shull, G. and Fabro, S.: Assessment of the teratogenic potential of trimethadione in the CD-1 mouse. Toxicol. Appl. Pharmacol. 51: 59-71, 1979.

Sanyal, M. K.: In vitro development of rat conceptus during organogenesis in embryo: Relationship of embryo-placental growth and differentiation of changes in components of culture medium. J. Exp. Emb. Morph. (In press).

Brown, N. A., Goulding, E. H., Fabro, S.: The effects of ethanol on rat embryonic development in a postimplantation culture system. Proceedings of the Fetal Alcohol Syndrome Workshop, 1980 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-04 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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	Head, Molecular Embryology Group	LRDT	NIEHS
OTHER:	P. E. Mansson	Visiting Fellow	LRDT	NIEHS
	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
Molecular Embryology Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline -

The objective of this project is to study the mechanisms involved in androgen mediated gene expression in the rat prostate and the seminal vesicle. 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 for this group of secretory polypeptides. The structural genes for these polypeptides have been prepared and cloned using the "tailing method" and the plasmid pBR322. Many of the plasmids containing structural genes for these androgen dependent polypeptides have been identified by hybrid arrest translation. Large pieces of DNA containing the chromosomal gene for one of the seminal vesicle proteins (SVS IV) has been identified in a rat gene library and is presently being purified.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Seminal vesicles and prostates were removed from mature CD rats. Seminal vesicle secretions were collected for preparation of purified proteins. Seminal vesicle proteins IV and V were purified by a combination of ion exchange chromatography and molecular sieve chromatography. Poly(A^+)-mRNA was prepared by affinity chromatography using oligo-dT cellulose and fractionated on 5-20% sucrose gradients in either 1% SDS or 70% formamide. The wheat germ in vitro translation system was used to characterize various poly(A^+)-mRNA fractions as well as identify various cloned plasmids which contain structural DNA information. ^{3}H-complementary DNA was prepared using reverse transcriptase. RNA-DNA hybridizations were carried out under standard conditions. Double stranded complementary DNA was again synthesized using reverse transcriptase. A variety of restriction endonucleases were employed to cleave the major ds cDNA's prepared from both prostate and seminal vesicle. The fragments generated with the restriction enzymes were analyzed by neutral agarose gel electrophoresis. ds cDNA was treated with S1 nuclease under conditions to cleave the single stranded "hairpin" loop at the 5' end. The ds cDNA was then "tailed" with either dT or dG residues using terminal deoxynucleotide transferase and annealed to plasmid pBR322 (previously tailed with dA or dC at the single Pst I site). These chimeric plasmids were mixed with Ca++ treated E. coli RR1. Tetracycline resistant colonies were selected and then tested on plates containing ampicillin (amp). The amp sensitive colonies were then grown on millipore filters and tested by colony filter hybridization with ^{32}P-cDNA probes enriched for prostate or seminal vesicles mRNA's. Colonies giving strong positive hybridization signals on the filters were selected, plasmid purified and the inserts characterized by mapping and hybrid arrest translation.

Purified DNA inserts from recombinant plasmids that contain the structural gene for SVS IV has been radiolabeled by nick-translation and used as a probe in Southern hybridization of endonuclease restricted total rat genomic DNA. This probe to SVS IV has also been used to screen a λ phage Charon 4A derivative containing 15 Kilobase fragments of rat genomic DNA.

MAJOR FINDINGS AND PROPOSED COURSE: When total poly(A^+)-mRNA from rat ventral prostate is analyzed by sucrose gradient centrifugation, a major peak of RNA (40% of the total) is observed at about 10S-13S. This RNA, having a 10S sedimentation rate can be fractionated into a light and heavy side. The light side is enriched in two poly(A)-mRNA referred to as γ and Δ mRNAs, while the main heavy side is enriched in poly(A^+)-mRNA referred to as β mRNA. By denaturing agarose gel electrophoresis, the size of prostate β mRNA and γ, Δ mRNA are determined to be 520 nucleotides (NT) and 450 NT, respectively. Δ and γ mRNA codes in a wheat germ translation system for a polypeptides of 14,000 and daltons 8,000 respectively. β mRNA codes, on the other hand, for a major polypeptide which is related to the major protein subunit of prostatein or prostate binding protein. Near the 13S region another major mRNA fraction

(900 NT) can be found which codes for a polypeptide of about 22,000 daltons and is also androgen dependent. This protein has been referred to as the α protein. Plasmids have been purified and insert DNAs for the four prostate polypeptides (α, β, γ, Δ) have been identified by hybrid arrest translation (see Z01 ES 70046-01 LRDT) and restriction mapping for these clones is presently underway.

A major 11S poly(A^+)-mRNA has been identified which comprises 40% of the total poly(A^+)-mRNA in the seminal vesicle. This 11S poly(A^+)-mRNA appears as two major bands in agarose gel electrophoresis under denaturing conditions. The size of the two poly(A^+)-mRNA bands are 620 NT (referred to as mRNAsvs IV) and 540 NT (referred to as mRNAsvs V). Poly(A^+)-mRNAs enriched for mRNAsvs V code (wheat germ) for a polypeptide of 18,000 daltons; similar in molecular weight but slightly larger than the seminal vesicle secreted protein IV. Likewise, the mRNAsvs V codes for a polypeptide slightly larger than the secreted protein V.

Double-stranded cDNA to poly(A^+)-mRNA 11S was prepared as described above. The two seminal vesicle synthetic genes were separated and preparatively isolated by gel electrophoresis using low melting agarose. The bands were removed, the agarose melted, and the DNA isolated by hydroxyapatide chromatography. Restriction maps were then generated for the two synthetic genes. Very interestingly, it appears as if the structural parts of these two seminal vesicle genes have considerable sequence homology, at least with respect to restriction enzyme cleavage sites.

ds $cDNA_{11S}$ was "tailed" and cloned in pBR322 similar to the prostate material except dG_5-dC tailing was used. This allows one to remove the inserted DNA using Pst I digestion. Clones containing the structural gene for SVS IV were identified by their size, DNA sequencing, restriction mapping, and hybrid arrest translation. Potential clones for SVS V have also been identified. One clone, labeled pSV IV-2, contains a full length insert of the structural gene for SVS IV. By partial DNA sequencing using the chemical method, the 5' -> 3' orientation and the coding and untranslated regions of the structural gene have been defined by comparison with the known amino acid sequence of SVS IV.

Other clones are presently under investigation to see if a recombinant plasmid can be found which contains an insert for the structural gene for SVS V. A partial Hae III rat genomic DNA library (generous gift of Prof. James Bonner) was screened for fragments of rat DNA in λ Charon 4A phage plaques. Approximately three million initial clones were screened and 11 positive signals were obtained using nick-translated insert from pSV IV-2 as probe. Presently, these 11 signals are undergoing secondary and tertiary screens to purify the individual phage plaques containing chromosomal DNA for SVS IV.

In the future, we hope to obtain a series of overlapping phage clones (from the 11 signals) and establish the linkage of SVS IV and SVS V in the genome

and the size and number of intervening sequences in the two genes. Nucleotide sequence information will be obtained on the flanking sequences and other regions that may be involved in androgen receptor binding sites and/or other potential specific regulatory macromolecules.

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., Carter, D. B., Silverberg, A. B., Tully, D. B. and Harris, S. E.: Isolation and partial purification of the major abundant class rat seminal vesicle poly(A^+)-messenger RNA. Nucleic Acid Res. 7: 1553-1565, 1979.

Silverberg, A. B., Tully, D. B., Mansson, P. E. and Harris, S. E.: In vitro translation of major abundant class poly(A^+)-mRNA from rat seminal vesicle. Arch. Androl. 4: 249-255, 1979.

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. (In press).

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. 15: (In press).

Carter, D. B., Silverberg, A. B. and Harris, S. E.: The effect of spironolactone on androgen dependent proteins of rat ventral prostate. J. Endocrinol. (In press).

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 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70047-04 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Prostate as a Model System to Study Normal and Abnormal Gene Expression in the Rat and Human*

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

PI:	D. B. Carter	Senior Staff Fellow	LRDT	NIEHS
OTHER:	K. Yamada	Visiting Fellow	LRDT	NIEHS
	S. E. Harris	Head, Molecular Embryology Group	LRDT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Molecular Embryology Group

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)

Two clones for structural genes coding for proteins secreted from the rat ventral prostate have been identified by hybrid arrest translation. The two cloned structural genes are currently being mapped with a variety of restriction endonucleases. Restricted recombinant plasmid is being electrophoresed on agarose gels and prostate insert fragments are isolated. The prostate inserts will be used for preparing radioactive probes which will be nick translationed and used to screen a chromosomal rat gene library carried in λ phage charon 4A. Similar protocols will be followed after isolation and identification of the human prostate structural gene for the major secretory protein (17,000 daltons) from human prostate.

(*Formerly, Regulation of Gene Expression During Differentiation)

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Poly(A^+)-mRNA from rat ventral prostate was fractionated by SDS-sucrose gradients and double-stranded complementary (cDNA) DNA was made to the abundant 10S-13S mRNA. After "tailing" the cDNA with deoxyadenosine residues using terminal deoxynucleotide transferase, the mixture was annealed to dT tailed plasmid PBR322 and E. Coli RR1 were transformed with the recombinant plasmid. Ampicillin sensitive/tetracycline resistant clones were assayed after plasmid purification by hybrid arrest translation of total poly(A^+)-mRNA hybridized with restriction endonuclease digested plasmid DNA isolated from the various clones.

MAJOR FINDINGS AND PROPOSED COURSE: Restriction maps of cloned structural genes coding for two of the major secretory proteins from rat ventral prostate have been generated. Purified inserts from these plasmids have been obtained from agarose gels. The size of the insert coding for a 12,000 dalton translation product is 1020. The size of the insert coding for 20,000 dalton translation product is 1400 base pairs and can be excised from the plasmid by the restriction endonuclease Hha I. Nick translated probes will be used to screen a rat gene library for the corresponding genomic sequences.

In addition, we have run a number of gels of prostatic secretions from normal human males and report a 17,000 dalton protein which is secreted by normal males in every case (10) looked at thus far. The structural gene coding for the major low molecular weight human secretory protein is a good candidate for cloning and would provide a unique marker for human secretory cells in tissue culture systems.

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 prostate 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. (In press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70060-07 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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 X (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline)

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, quantity, and morphology of ova obtained from the female reproductive determinations of the various follicular classes. Pharmacological, biochemical, physiologic, and morphologic 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.

Scanning electron microscopy (SEM) of the genital tract of these females has revealed surface ultrastructural abnormalities which may precede neoplastic changes; SEM may provide a tool for early detection of neoplastic change in the affected tissues. This technique was also used to demonstrate hormone-related changes in genital tract tissue during the estrous cycle and with the onset of puberty. The latter study coupled with determinations of estrogen receptor levels and enzyme activities suggests that hormone responsiveness is manifested earlier in the vagina than in the uterus. Alterations in the

location of squamocolumnar junction, abnormal uterine surfacr architecture, as well as vaginal polyps have been determined by this approach. Both male and female offspring of DES-treated mice had abnormal immune function.

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). Additionally, SEM studies of the male genital tract have been undertaken to establish sensitive morphological parameters of cellular abnormalities. 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. Similar altered patterns in the uterine luminal fluid were consistent with altered morphology.

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. Moreover, 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. This same activating system utilizing peroxidase was determined in the hamster kidney, a non-genital target tissue for DES. 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 as well as prostaglandin synthetase.

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 carcinogenetic changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PUBLICATIONS

McLachlan, J. A.: Transplacental effects of diethylstilbestrol in mice. In Perinatal Carcinogenesis, NCI Monograph 51, 1979, pp. 67-73.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-04 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

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	L. Levy	Research Chemist	ECB	NIEHS
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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. 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 have an altered pattern of receptor depletion/replenishment. Receptor resynthesis, RNA polymerase activities, DNA polymerase activities and DNA synthesis are also being investigated.

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 alphafetoprotein, 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 potential carcinogenic and hormonal activity is being tested.

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 resynthesis 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 these compounds do not significantly stimulate progesterone receptor synthesis. This result may in part be 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.: Estrogen action in the mouse uterus: Characterization of the cytosol and nuclear receptor systems. Endocrinology 104: 1324-1332, 1979.

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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, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70080-07 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.3	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)

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 gain more reliable extrapolation of laboratory data to man and improve estimates of human risk. The following studies are ongoing: (1) defining the role of germ cell DNA repair as an indicator and modifier of toxicity; (2) modeling the various factors which affect testicular toxicity; and (3) investigating the toxication-detoxication of polycyclic aromatic hydrocarbons in gonadal and prostate tissues using both in vitro perfusion and cell free preparations. To date, selected mutagens, trace metals, the nematocide, dibromochloropropane diesel, trimethylamidoborane, and tetrachlorodibenzo-p-dioxin have been studied. Alkaline elution analysis of germ cell DNA, unscheduled DNA synthesis, and determination of germ cell nicotinamide adenine dinucleotide level appear to be reliable indicators of DNA damage and unscheduled DNA repair activity, as well as predictors of toxicity. The interrelationship of the blood-testis barrier, toxication-detoxication reactions, and DNA repair have been described.

PH-0040
(Rev. 10-78)

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) DNA repair. Effects of 1,2-dibromo-3-chloropropane (DBCP) on spermatogenic cell DNA were investigated by labeling spermatogonial cells of 15 to 30 day old rat with repeated i.p. administration of tritiated thymidine. Following DBCP administration, the spermatogenic cells were isolated from minced tubules by 0.1% collagenase digestion, followed by albumin gradient centrifugation. DNA single strand breaks, as a function of time, were estimated by alkaline elution analysis elution profiles.

(B) In vitro perfusion of rat testis. Adult Sprague-Dawley rats were treated with a single oral dose of TCDD (10 µg/kg body weight). Seventy-two hours following the treatment, a rat testis was quickly excised and the testicular artery cannulated with an 80 micron glass capillary tube attached to polyethylene tubing. An oxygenated Krebs-Ringer bicarbonate solution containing 3% albumin (BSA fraction V) was used to perfuse testes at 20 ml/g/hr. Benzopyrene 7,10-^{14}C (3.6×10^{-6}M) was perfused and BP metabolites in the organic extractable phase were determined by a combination of differential solvent extractions and high pressure liquid chromatography (HPLC) and liquid scintillation spectrometry. Viability of the perfused testes were assessed by glycolytic activity, testicular tissue ATP levels, and morphological studies by electron microscopy.

(C) Studies of polycyclic aromatic hydrocarbon activating and deactivating enzyme systems. Benzo(a)pyrene hydroxylase (AHH), epoxide hydrase (EH), glutathione transferase (GSH-T), and cytochrome P-450 were measured in both testicular and prostatic tissues. The various factors affecting the specific activities of these enzymes in the testes and prostate glands studied include pretreatment with tetrachlorodibenzo-p-dioxin (TCDD), diesel exhaust, and DBCP.

(D) Effects of prenatal exposure to environmental chemicals on the testicular development and function of F_1 male offsprings. Day-bred Sprague-Dawley female rats were treated (on day 10, 11, 13, and 14 of gestation) with daily oral doses of corn oil (control), 0.25, 0.5, 1.0, or 2.0 µg/kg body weight TCDD. Pregnant females were allowed to litter at term and F_1 males were allowed to wean and become adults (70 days old). Leydig cell function; plasma FSH, LH and testosterone (T); and tissue levels of T and 5-α dihydrotestosterone (DHT) were studied. In addition, testicular weights and histology were statistically evaluated.

(E) In vivo assessment of fertility. Fertility was assessed using the serial mating technique. Fertility profiles were drawn relating infertility to the type of spermatogenic cells affected. Effects of trimethylamidoborane (TMB) were evaluated as the extension of sodium borate studies. A single intraperitoneal dose of 150, 300 and 600 mg/kg was administered to CD-1 male mice and the males were immediately subjected to the serial mating to evaluate the reproductive effects of this compound.

MAJOR FINDINGS AND PROPOSED COURSE: (A) DBCP has been extensively used in agriculture and linked to an increased incidence of male infertility among workers in processing plants. Sterility was associated with low sperm counts and a concomitant elevation of plasma FSH, accompanied by germinal aplasia. Recovery from the DBCP-induced germinal aplasia is uncertain at this time. Previously, we have shown that a single i.p. administration of DBCP (80, 100, 120 and 130 mg/kg body weight) induced spermatogenic cells to undergo linear unscheduled DNA synthesis for up to 8 hrs. In the current studies, the alkaline elution technique was utilized to correlate the time course of elutable DNA single strand breaks. Following the single highest dose of DBCP (130 mg/kg) treatment, the time course of elutable DNA at 3, 6, 16, and 24 hrs were 30, 65, 95, and 56% of controls. These results parallel the time course of unscheduled DNA synthesis. Because DBCP lacks reactive moiety, single strand DNA breaks suggest that DBCP is metabolized to products which interact with the nucleophic sites of DNA. The reactive metabolite (or metabolites) of DBCP is unknown at the present time. Further studies are in progress.

(B) We have previously reported the existence of aryl hydrocarbon hydroxylase (AHH), epoxide hydrase (EH), and glutathione transferase (GSH-T) in subcellular fractions of rat testis. The in vitro cell free system, however, does not represent the biological complexity of the intact organ. Therefore, we have extended the study of benzo(a)pyrene using the isolated perfused testis technique. BP metabolites in the effluent and the tissue compartment of testis were analyzed using HPLC. Comparison of BP metabolites in control and TCDD-induced testes showed that the total amounts of BP metabolites recovered during the 60 min perfusion were 18.3 and 26.2% of total radioactivity. The pattern of BP metabolites in the control testes demonstrated that the major metabolites in the organic extractable phase were 9,10-diol (52.1%) and 7,8-diol (16.3%). Smaller amounts of 3-OH (9.2%), quinones (8.6%), and 4,5-diol (4.4%) were also present.

This pattern of BP metabolites contrasts with that observed in rat livers or lungs where phenols and quinones are the primary metabolites. TCDD treatment increased BP metabolism with concomitant increase in the testicular AHH activity. The magnitude of various BP metabolites in TCDD-treated rats ranged from 1.5 to 2.8 times control except 4,5-diol which was not altered by TCDD treatment. Mixed function oxidase(s) in rat testis appear to preferentially epoxidate the 9,10- and 7,8- position of BP. Since 7,8-diol is the precursor of the potent carcinogen, 7,8-diol 9,10-epoxide, the TCDD-induced shift in metabolic products could be significant in BP-induced mutagenic and/or carcinogenic effects.

(C) Induction of AHH in prostate glands following a single oral dose of TCDD (10 µg/kg body weight) was previously shown in our laboratory to be 200 fold. TCDD-induced cytochrome P-450 forms were identified by SDS polyacrylimide gel electrophoresis. The results demonstrated that both 55,000 and 57,000

dalton cytochrome P-450 were significantly increased in TCDD-treated prostate glands. Furthermore, a single pretreatment of a DNA damaging agent (procarbazine) at 200 mg/kg body weight 24 hr prior to a single oral dose of TCDD, potentiated the induction of AHH 1,000 fold. The greater induction of AHH by TCDD under these conditions is probably due to greater accessibility of the AHH loci of the prostatic DNA to TCDD. HPLC analysis of BP metabolites produced by rat prostate glands following a single oral dose of TCDD was used to determine the kinetics of the reaction. The initial rate of formation of phenols, quinones and dihydrodiol in the prostate glands of TCDD-treated animals was increased to 394, 251, and 32 times that of the controls. The rate of 7,8-diol formation was three fold greater than that of either 4,5-diol or 9,10-diol formation. Thus, the magnitude of AHH induction in prostate glands by TCDD far exceeds that reported for any other organs, including liver.

Sprague-Dawley rats were exposed to diesel emissions for 20 hrs daily for 42 days at 14.2 ppm (v/v) total hydrocarbon. At various times, AHH, EH and GSH-T activities were determined in lung, liver, testis and prostate glands. The results indicated that, although liver exhibited the greatest overall AHH activity among the organs tested, the percent increase over control values was highest in the prostate glands. On day 15 of exposure, prostate AHH activity had increased 4.5 times control and remained elevated throughout the entire exposure period. In contrast, AHH induction in liver and lung occurred on day 33 of exposure; the maximum AHH induction (30-40%) was observed on day 42. EH and GSH-T activities were unchanged by exposure to diesel exhaust.

(D) Prenatal exposure (gestational days 10-14) to varying doses of TCDD demonstrated that F_1 male leydig cell function was affected *in vitro*. Testosterone (T) secretion following LH administration was lower than control. LH response in control, 0.25, 0.5, and 1.0 µg/kg TCDD dose groups were 7.7 ± 2.2 µg, 5.7 ± 1.8 µg, 5.4 ± 1.3 µg and 3.6 ± 1.6 µg T/hr/testis, respectively. However, if the testosterone secretion rate is calculated on the basis of g testicular weight, there was no significant difference. Testicular weights of prenatally exposed F_1 males were significantly reduced and the magnitude of testicular weight decrease was dose dependent. Testicular weights of control and 0.25, 0.5 and 1.0 µg/kg TCDD dose groups were 3.52 ± 0.24 g, 3.22 ± 0.32 g, 2.99 ± 0.31 g and 2.81 ± 0.23 g, respectively. Histological examination revealed a significant number of aplastic seminiferous tubules in the 0.5 and 1.0 µg/kg groups. However, there were also normal seminiferous tubules with mature spermatozoa. Thus, the functional consequence of spermatozoa from TCDD-treated group is uncertain at this time and needs further evaluation.

(E) *In vivo* assessment of fertility. TMB is an organoboron compound which has a high therapeutic index for tumor-bearing mice. A single dose (i.p.) of TMB doses at 150, 300 and 600 mg/kg had no antifertility effect during the six-week period following treatment. However, histological examination revealed some seminiferous tubular damage in the highest dose group.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Toxicological studies of a target organ, such as the testis, or male accessory gland, 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 cell, 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. The biological significance of AHH induction in prostate glands needs further study with respect to polycyclic aromatic hydrocarbon-induced carcinogenesis.

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

Lee, I. P. and Suzuki, K.: Induction of unscheduled DNA synthesis in mouse germ cells following 1,2-dibromo-3-chloropropane (DBCP) exposure. Mutat. Res. 68: 169-173, 1979.

Dixon, R. L. and Lee, I. P.: Pharmacokinetic and adaptation factors involved in testicular toxicity. Fed. Proc. 39: 66-71, 1980.

Lee, I. P., Suzuki, K., Lee, S. D. and Dixon, R. L.: Aryl hydrocarbon hydroxylase induction in rat lung, liver, and male reproductive organs following inhalation exposure to diesel emission. Toxicol. Appl. Pharmacol. 52: 181-184, 1980.

Lee, I. P., Suzuki, K., Mukhtar, H. and Bend, J. R.: Hormonal regulation of cytochrome P-450-dependent monooxygenase activity and epoxide-metabolizing enzyme activities in testis of hypophysectomized rats. Cancer Res. 40: 2486-2492, 1980.

Tyrer, H. W., Cantrell, E. T., Horres, R. and Lee, I. P.: Benzo(a)pyrene metabolism in mice exposed to diesel exhaust. I. Uptake and distribution. Environ. Interntl. (In press).

Lee, I. P. and Nagayama, J.: Metabolism of benzo(a)pyrene by the isolated perfused rat testis. Cancer Res. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70085-03 LRDT

PERIOD COVERED
October 1, 1979 to September 30, 1980

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

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Germ Cell Toxicity Group

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) MINORS ☐ (a2) INTERVIEWS

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

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 extremely useful test 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. 10-76)

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 80 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 31% (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 twelve couples presently with pregnancies or delivering during the study gave positive fertilization results in the in vitro test-system. The underlying mystery of any semen samples is the fertilizing ability of the sperm. Although we cannot morphologically distinguish between "fertile" and "infertile" sperm, the data pertaining to the validity of this test suggest that we can 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 91%. 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 optimal results and those ova were equal to fresh ova in fertilizability.

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 ultrastructurally and for lectin binding for possible membrane damage 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 and the comprehensive questionnaire presently being completed by a large group of males should provide the largest data base available for evaluating the relationship of 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. J. Reprod. Med. (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).

ENDOCRINE LABS OF MADISON - Madison, Wisconsin 53707
(NIEHS NO1-ES-6-2111)

TITLE: Effects of Environmental Agents on Male and Female Reproductive Tract Function

CONTRACTOR'S PROJECT DIRECTOR: Karen MacKenzie, Ph.D.

PROJECT OFFICERS (NIEHS): G. W. Lucier, Ph.D., Acting Chief, Laboratory of Organ Function and Toxicology
J. A. McLachlan, Ph.D., Head, Transplacental Toxicology Group, LRDT

DATE CONTRACT INITIATED: March 20, 1976

CURRENT ANNUAL LEVEL: $130,838

PROJECT DESCRIPTION

OBJECTIVES: A series of experiments are described which will provide information on the effects of 36 selected xenobiotics (10 per annum) on the fertility of mice (male and female) following prenatal exposure, and determine if levels of reproductive hormones have deviated from the norm or if a pathologic condition exists in mice whose reproductive capacity has been altered. Information will be obtained on the possible developmental toxicity of these chemicals, including transplacental carcinogenesis.

METHODS EMPLOYED: The approach that we are using to assess transplacental toxicity involves three phases of investigation. Phase I involves a preliminary screen to determine the "critical dose", which is defined as the highest dose that does not cause significant fetotoxicity or teratogenicity. Pregnant CD-1 mice are used as the test animal and chemicals are administered on days 10 through 16 of gestation. When generating the value for the "critical dose," we attempt to collect meaningful teratogenic and fetotoxic data so that maximum experimental information can be obtained from the protocol. In the teratogenicity studies, two-thirds of the fetuses (19 days of gestation) from each litter are fixed in 95% methyl alcohol and examined for skeletal alterations. One-third of the fetuses are preserved in Bouin's fixative, sectioned, and examined for internal anomalies. Autopsy data include determinations of corpora lutea, implants, resorptions, viable fetuses, fetal body weight, and sex ratio.

Dose schedules for animals used in Phase II are; controls, one-half of the critical dose, and one-tenth of the critical dose. If no "critical dose" is established, doses of one-half and one-tenth the LD_{50} are employed. In Phase II studies, 24 pregnant females are used in each group and are administered vehicle or vehicle plus chemical on days 10 through 16 of gestation. One group of animals receives a dose of DES (10 mg/kg on days 10-16), which

significantly reduces fertility of both male and female offspring and this group is used as a positive control. Parameters evaluated in the initial Phase II studies include fertility, gestation, viability, survival, and lactation indices. Necropsy examinations of F_1 males are made at eight weeks, six months, 12 months, and a terminal necropsy is also performed. F_1 males are examined at seven weeks and again at the termination of the experiment. Tissues examined for histological alterations include liver, kidney, lung, small intestine, spleen, thymus, pituitary, adrenal, thyroid, parathyroid, gonads, uterus, epididymis, cervix, vas deferens, vagina, seminal vesicles and prostate. All tissues are checked for gross lesions. Fertility of at least 20 F_1 males are evaluated at eight weeks and again at eight months of age. Two virgin females (3-10 weeks old) are placed with each male every five days for 25 days (total of 10 females per male). Twelve days after removal, the females are weighed, laparotomized and the number of fetuses (alive, dead, or abnormal) and resorptions recorded. Fertility of F_1 females is determined by a lifetime forced-breeding study. Fifty F_1 females are selected randomly from each treatment group and each female is cohabited with one proven breeder male. The F_1 females remain with the males and are allowed to deliver at term. The number of F_2 young (alive and dead) are recorded and the young examined for gross anomalies. Litters are examined again on days 4 and 20. New breeder males are replaced at 180-day intervals. Plasma samples are taken prior to necropsy for analysis of progestins, androgens, and estrogens in groups of treated animals exhibiting deficits in reproductive tract function. The estrous cycle is monitored in eight week old F_1 females.

Although emphasis is placed on reproductive tract function of F_1 offspring, it would seem wasteful and narrow-minded to ignore manifestations of impaired organ function of other systems. Accordingly, the offspring are monitored for behavioral parameters, immunological competence, transplacental carcinogenesis, and cardiovascular function.

The objectives of Phase III are to determine dose-response relationships of chemicals exhibiting transplacental toxicity, to elucidate mechanisms of toxicity, to characterize structure-activity relationships, and to define the role of perinatal pharmacokinetics in transplacental toxicity. Of course, the protocols for Phase III studies cannot be developed until at least portions of Phase I and Phase II studies are completed. Many of the Phase III studies are conducted at the NIEHS.

MAJOR FINDINGS AND PROPOSED COURSE: Chemicals investigated or planned for study include DES, TCDD, DES + TCDD, Zearanol, Zearalenone, Mirex, Kepone, DDE, Methoxychlor, Hexabromobiphenyl (6-BB), Hexachlorobiphenyl (6-CB), Tetrachlorobiphenyl (4-CB), DMBA, 3-Methylcholanthrene, Benzpyrene, Dichloropropene, Phosphamidon, Dicrotophos, Atrazine, Aldicarb, and Carbaryl. Completed protocols for reproductive capacity following treatment with these chemicals are not available since these are lifetime studies. The fetotoxic and/or teratogenic dose has been established or will be established for each of these compounds in the CD-1 mouse. For example, Zearanol, a synthetic Mycotoxin analog, was shown to cause skeletal abnormalities at a dose of 300 mg/kg/day on days 10-16 of gestation. The estrogenic potency of this compound was determined

to be approximately 1/600 that of DES. However, reproductive capacities of these offspring were unaffected. Localization of Zearanol/Zearalenone in specific fetal tissues will be performed by radioimmunoassay procedures.

Marked and unusual behavioral changes were observed in offspring from 4-CB treated (32 mg/kg/day) pregnant mice by two to three weeks after parturition. Increased locomotor activity and general restlessness were noted in approximately half of the surviving litters. General symptoms included characteristic jerking or rotational movement of their heads. There were also episodes of constant circling movement which seemed to be initiated by a loud noise. All offspring in an affected litter exhibited behavioral alterations and the symptoms were evident at least through eight months of age. Affected animals gained weight at approximately the same rate as controls and the survival rates of rotating and non-rotating offspring in the treated group were not different. Moreover there was no correlation between hydronephosis and behavioral alterations and the occurrence of hyperactivity was not sex-related. The complexity of the symptomology indicates damage to the central nervous system. Although conventional histological techniques did not find microscopic alteration, electron micrographs revealed CNS outgrowths into the peripheral nervous system. This lesion resembles those observed in the Werdnig-Hoffman syndrome. Foster mother experiments demonstrated that the effect is related to prenatal exposure to 4-CB.

Tissue distribution studies of ^{14}C-4-CB in CD-1 mice revealed some important findings. We observed that only very low levels of ^{14}C-4-CB were detected in weanlings following exposure to ^{14}C-4-CB via both placental transfer and suckling. Radioactivity was not detectable in eight week-old offspring indicating that behavioral alterations occur in the absence of the chemical. These studies also revealed that newborn tissues had, in general, higher 4-CB concentrations at parturition than did maternal tissues. This finding may help explain the occurrence of fetotoxicity and teratogenicity in 4-CB treated groups whereas no such effects were found in 6-CB and 6-BB treated animals.

It also appears that mice exposed to 4-CB prenatally have decreased reproductive capacity.

Studies with the polycyclic aromatic hydrocarbon (PAH) dimethylbenzanthracene (DMBA), an environmental carcinogen, have shown that gestational treatment with as little as 1.25 mg/kg results in absence of germ cells in both male and female offspring at seven weeks of age. Other abnormalities were not noted; these effects could not be induced with other PAHs, 3-methylcholanthrene or benzpyrene at doses 50 fold higher than that for DMBA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Environmental chemicals can cause reproductive toxicity in mammals which is manifested in decreased fertility. However, these chemicals might affect male and female reproductive processes differently since oogenesis is an entirely prenatal event and spermatogenesis occurs throughout adult life in most

eutherian mammals. The possibility exists that lesions of the reproductive tract which occur in utero might result in long-term impairment of the reproductive tract in females and have a totally different effect on the male reproductive process.

The objective of the proposed study is to determine the effects of environmental chemicals on the reproductive capacity of male and female mice. Reproductive capacity is to be evaluated by the repetitive forced breeding method for females and by a serial mating technique for males. Since those animals are held for 18 months, developmental toxicities of other organ systems are noted including the CNS, immune and cardiovascular systems. The potential for transplacental carcinogenesis is also noted.

These studies are done in such a way as to support existing programs in the Laboratory of Reproductive and Developmental Toxicology concerned with developmental toxicity and transplacental toxicity. The purpose of the contract is two-fold; to identify environmental chemicals that have potential developmental toxicity emphasizing reproductive tract lesions; and to identify chemicals to Intramural scientists that are toxic and need to be studied from a mechanistic viewpoint.

PUBLICATIONS

Chou, S. M., Miike, T., Payne, W. M. and Davis, G. J.: Neuropathology of spinning syndrome induced by prenatal intoxication with a PCB in mice. Ann. N. Y. Acad. Sci. 320: 373-395, 1979.

Chou, S. M., Miike, T. and Davis, G. J.: Experimental induction of Werdnig-Hoffman-type neuroglial peninsulas by prenatal PCB intoxication. In Tsubaki, T. and Toyokura, Y. (Eds.): Amyotrophic Lateral Sclerosis. Tokyo, University of Tokyo Press, 1979.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

During Fiscal Year 1980, the National Advisory Environmental Health Sciences Council reviewed 488 applications assigned to NIEHS as primary or secondary assignee. This represents an overall decrease of twenty-three applications from Fiscal Year 1979. One hundred and six awards were made; 88 regular research grants, 1 Environmental Health Sciences (EHS) Center (competing), 4 Research Career Development Awards; 4 Institutional National Research Service Awards (NRSA); and 9 Individual NRSAs. No new or competing applications were funded in the Marine and Freshwater Biomedical (MFB) Centers program, the Mid-Career Development Award (MCDA) program or in the Senior Fellowship program. These new and competing awards plus the non-competing awards brought the 1980 total awards to 423 active grants, a decrease of 37 awards from Fiscal Year 1979.

The Young Environmental Health Scientist (YES) program continues to be an active and viable program for the NIEHS. This award mechanism was recently extended to all awarding Bureaus, Institutes and Divisions (BIDs) of the NIH, new guidelines were developed and the program was renamed the New Investigator Research Award (NIRA). The new guidelines provide uniformity for the management and awarding of this support mechanism within all awarding units.

NIEHS is currently supporting 42 NIRA grants in 35 institutions. This represents an increase of 21 awards over Fiscal Year 1979 and is an indication of the acceptance of this program by the scientific community as a mechanism for support of new faculty who have not yet received support through a regular research grant (R01).

The recent meeting of the EHS Centers' Program Directors at Vanderbilt, June 10-11, demonstrated the importance of the Centers in studies of Environmental Health and the ability of the Center Directors to respond to environmental health emergencies. Of the nine Centers, six had already initiated or were planning to initiate studies on the health effects resulting from the eruption of Mount St. Helens. These studies included, for example, ash analysis, fluorid content, inhalation studies of respirable ash particulates, diet studies, tissue reactions, and studies of human lung tissues from victims of the eruption. The twice-yearly meetings of NIEHS staff with the Center Directors greatly facilitate the exchange of information and keeps the NIEHS staff informed of ongoing research activities within these Centers.

No new MFB Centers were funded in Fiscal Year 1980, although interested institutions were encouraged to submit applications.

Program Planning activities have expanded to include the collection of data on Environmental Health Sciences research supported by other BIDs and the level of support in areas of programmatic interest to NIEHS. This information, along with NIEHS supported research and funding levels, will be utilized in future program planning and funding decisions for the Extramural Program.

Computerization of the EP grants is underway through a contract with Computer Sciences Corporation. Training and Fellowship related data have been pulled together and should be available on the computer for program administrative use by the beginning of Fiscal Year 1981. Other ongoing activities include preparation of a scientific vocabulary for coding of research grants and testing of the proposed coding system.

NIEHS Staff and the NAEHS Council have undertaken a study of the Institute support of research not clearly relevant to the programmatic goals of the Institute. As a result of this study, new referral guidelines have been developed for assignment of research applications. Approved applications are being reassigned to other BIDs and NIEHS will begin phasing-out those grants not clearly relevant. This initiative was begun to permit a better focused program and funding of grants in areas of major responsibility as identified and recommended by the Program Planning Groups.

RESEARCH MANPOWER DEVELOPMENT SECTION

General

The NIEHS extramural training program operates under the authority of the National Research Service Awards Act authorizing both pre- and postdoctoral training. Both institutional and individual (fellowship) awards are made under this program. In the past year the following important changes have been made in implementing this Act.

- both pre- and postdoctoral stipends have been raised substantially
- short-term training has been authorized for giving medical students exposure to research
- a senior fellowship program has been developed to support sabbatical leaves of absence and retraining

It is the goal of the NIEHS Training Programs to train individuals for careers generating new knowledge in the biomedical aspects of environmental health sciences. These individuals will, through research, promote better comprehension of current and potential hazards to man of exposure to environmental agents.

The following four areas of training fall within the scope and authority of the NIEHS:

Environmental Toxicology: Training in this area focuses on didactics and research experiences in toxicological principles which determine the effects on organs and tissues of exposures to environmental agents. Pharmacokinetic and pharmacodynamic factors, cellular and molecular mechanisms of action, synergism, species variability in toxic response, and test development, design and interpretation are all facets of this training area. Graduates are qualified to pursue careers in toxicology in academia, industry or government.

Environmental Pathology: Training in this area focuses on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees and fellows 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. Graduates are qualified to become members of research teams involved in chemical risk evaluation using experimental models where their primary roles will be to lead in the pathological phases of research and to assist in experimental design and interpretation, and/or initiate independent research on the functional and morphological consequences of environmentally induced diseases.

Environmental Mutagenesis: Training in this area emphasizes the application of the basic principles of genetics and biochemistry to applied studies aimed at assessing the potential genetic hazards to man of exposure to environmental chemicals. Research on the basis for chemically induced mutations is stressed. Graduates enter careers aimed at understanding hazards and developing predictive tests in this field.

Environmental Epidemiology and Biostatistics: Training in epidemiology teaches the utilization of statistical and mathematical tools to assist in the identification of environmental diseases in human populations. The focus is on non-infectious disease epidemiology with an emphasis on the identification of causes of environmental disease. Biostatistics training teaches the application of mathematical and statistical tools to assist environmental health scientists in experimental design and interpretation. These programs provide research epidemiologists and statisticians to analyze data from human or animal populations to determine the potential human hazards of exposure on environmental agents.

The following institutions are supported for training in these areas:

ENVIRONMENTAL TOXICOLOGY

	INSTITUTION	DIRECTOR
1.	Johns Hopkins University School of Hygiene & Public Health Division of Toxicology Department of Environmental Health Sciences	Robert J. Rubin (Pre/Post)
2.	University of California, Davis Department of Environmental Toxicology	James N. Seiber (Pre/Post)
3.	University of Cincinnati Department of Environmental Health	Paul B. Hammond (Pre/Post)
4.	Duke University Medical Center Physiology and Pharmacology Department	Daniel Menzel (Post only)
5.	University of Wisconsin Bacteriology Department	Ronald Hinsdill (Post only)
6.	Massachusetts Institute of Technology Nutrition and Food Science Department	Gerald Wogan (Pre/Post)
7.	University of Illinois Biology & Entomology Department	Robert Metcalf (Pre/Post)
8.	Vanderbilt University School of Medicine Department of Biochemistry	Robert Neal (Pre/Post)

9.	Medical College of Wisconsin Department of Pharmacology	James Fujimoto (Pre/Post)
10.	University of Mississippi Medical Center Department of Pharmacology and Toxicology	A. Wallace Hayes (Pre/Post)
11.	North Carolina State University Department of Entomology	Frank E. Guthrie (Pre/Post)
12.	Children's Hospital Research Foundation Pediatrics Department	Ernest Zimmerman (Pre only)
13.	University of Rochester Pharmacology and Toxicology Department	Victor G. Laties (Pre/Post)
14.	Duke University School of Medicine Pulmonary Medicine Division	William Lynn (Pre/Post)
15.	Cornell University Department of Entomology	Chris Wilkinson (Pre/Post)
16.	New York University Department of Environmental Medicine	Morton Lippman (Pre/Post)
17.	Albany Medical College Center of Experimental Pathology and Toxicology	Rajender Abraham (Pre/Post)
18.	Michigan State University College of Osteopathic Medicine Carcinogenesis Laboratory	J. Justine McCormick (Post only)
19.	New York University Medical Center Department of Environmental Medicine	Roy Albert (Pre/Post)
20.	Purdue University School of Pharmacy & Pharmacology Sciences	John E. Christian (Pre only)
21.	Oregon State University Environmental Health Sciences Center	Ian J. Tinsley (Pre/Post)
22.	University of Kansas Medical Center College of Health Sciences	Curtis Klassen (Pre/post)
23.	Thomas Jefferson University Jefferson Medical College	C. Paul Bianchi (Post only)
24.	Yale University School of Medicine	Margaret Hitchcock (Post only)
25.	Medical College of Virginia Department of Pharmacology	Albert Munson (Pre/Post)

26.	University of Texas Health Sciences Center Medical School & School of Public Health	Sheldon Murphy (Pre/Post)
27.	University of Arizona Health Sciences Center	J. Wesley Clayton (Pre/Post)
28.	Johns Hopkins University School of Hygiene & Public Health	Zoltan Annau (Pre/Post)
29.	Utah State University Toxicology Program	Joseph Street (Pre/Post)
30.	University of Michigan School of Public Health	Herbert Cornish Pre/Post)
31.	Roswell Park Memorial Institute New York State Department of Health	Harold Box (Pre/Post)
32.	Dartmouth College Dartmouth Medical College	Roger Smith (Pre/Post)

ENVIRONMENTAL EPIDEMIOLOGY AND BIOSTATISTICS

1.	University of North Carolina Department of Biostatistics	Lawrence Kupper (Pre/Post)
2.	Harvard University Department of Epidemiology	Richard Monson (Pre/Post)
3.	University of North Carolina Department of Epidemiology	Herman Tyroler (Pre/Post)
4.	Florida State University Department of Statistics	Fred Leysieffer (Pre/Post)
5.	University of Cincinnati Department of Environmental Health	Ralph Buncher (Pre/Post)
6.	University of Illinois School of Public Health	Henry Gelfand (Pre/Post)
7.	University of Pennsylvania School of Medicine	Anita Bahn (Pre/Post)
8.	Yale University School of Medicine Department of Epidemiology and Public Health	Jan Stolwijk (Pre/Post)
9.	University of California - Los Angeles School of Public Health	Roger Detels (Pre/Post)

ENVIRONMENTAL PATHOLOGY

1.	University of North Carolina Department of Pathology	Joseph Grisham (Post only)
2.	University of California, Davis Veterinary Pathology Department	Donald Dungworth (Post only)
3.	University of Washington, Seattle Department of Pathology	N. Karle Mottet (Pre/Post)
4.	Washington University, St. Louis Department of Pathology	Michael Lieberman (Pre/Post)
5.	University of Wisconsin Pathology Department	James Allen (Pre/Post)
6.	State University of New York--Stony Brook Department of Pathology	Philip Kane (Pre/Post)
7.	Mount Sinai School of Medicine Department of Community Medicine	William Nicholson (Post only)
8.	University of California, San Francisco Medical Center, Department of Pathology	Ed Smuckler (Pre/Post)

ENVIRONMENTAL MUTAGENESIS

1.	University of California, Berkeley Department of Biochemistry	Stuart Linn (Pre/Post)
2.	Case Western Reserve University School of Medicine Department of Radiology	Helen H. Evans (Post only)

In addition, 36 postdoctoral fellowships were supported in environmental toxicology and two in mutagenesis.

The training program budget has allowed substantial growth in the past five years as indicated below. This reflects a concern that an adequate supply of trained scientists be provided for implementing Federal initiatives in Chemical safety and health.

	FY 76	FY 77	FY 78	FY 79	FY 80
Budget ($1000's)	$1,745	$4,194	$4,568	$4,568	$6,568
Students Supported Pre/Post	115/79	182/149	179/176	209/177	256/201

The Mid-Careet Development Award in Environmental Toxicology was developed in response to a National Advisory Environmental Health Sciences Council recommendation about two years ago. The purpose of the award is to attempt to tap the perceived manpower pool of individuals with good, basic science training in fields ancillary to toxicology and allow these indivividuals to train in toxicology. It is hoped that these people will then continue their careers in toxicology research. Therefore, the program was set up to provide tutorships and research experiences for individuals under the guidance of established toxicologists to allow the individuals to receive up to three years of training and experience in research problems in this field. We are supporting three such individuals.

The Senior Fellowship Program is brand new and is designed to provide opportunities for experienced scientists to (1) make changes in the direction of their research careers, (2) to broaden their scientific background, (3) to acquire new research capabilities, or (4) to enlarge their command of an allied research field. The objective of the award is to enable an individual beyond the new investigator stage to take time from regular professional responsibilities for the purpose of increasing their capabilities to engage in health-related research. Normally these awards are for periods of twelve months but they are allowed for up to twenty-four months.

A comprehensive survey of supply/demand and need for environmental toxicologists is underway. In mid-1979, the NIEHS let a contract, entitled "Evaluation of the NIEHS Environmental Toxicology Training Program" (Contract No. NO1-ES-9-0005), which will assess the comparability between supply and demand for individuals with specific skills in environmental toxicology and their graduates and a survey of employers of environmental toxicologists. In addition, the impact of the NIEHS training programs on these factors will be determined. The contract is expected to run for two years and will be utilized as a major tool for guiding future thrusts of the NIEHS Extramural Training Program.

New York University

Toxicology. Research in toxicology includes identification of environmental toxicants; qualitative and quantitative definition of their physiological, behavioral, biochemical and morphological effects in man and animal models; and methodologies for determining their distributions and concentrations in man and various environmental matrices.

Investigation of sulfur dioxide and sulfite metabolism and toxicity has continued. Recent efforts have concentrated on the refinement and metabolic characterization of a sulfite oxidase-deficient rat model. Experiments are underway using this model to evaluate the possible fetotoxicity and teratogenicity of sulfite, as well as the previously reported sulfite induction of thiamine deficiency and anemia.

Another aspect of this project deals with the separation and identification of rabbit plasma proteins which are lysed by sulfite to form S-sulfonate compounds. Plasma containing ^{35}S-sulfonated protein was fractionated initially on Sephadex and then further separated and characterized by disc electrophoresis. Radioactivity was associated with albumen and with several other proteins yet to be identified.

Inhalation on exposures of non-smoking, healthy human volunteers to H_2SO_4 have been completed. Respiratory mechanical function was assessed by body plethysmography, partial forced expiratory maneuver and nitrogen washout before and ½, 2 and 4 hours after the H_2SO_4 exposure. A ^{99m}Tc tagged Fe_2O_3 aerosol was inhaled ~10 min before each H_2SO_4 exposure.

The results of this study, and those of coordinate animal studies, indicate that chronic H_2SO_4 exposures at the levels used could produce persistent changes in previously healthy individuals, and exacerbate pre-existing respiratory disease.

Comparable studies on volunteers with asthmatic histories and/or chronic bronchitis are underway in order to determine if such individuals may represent a more sensitive subpopulation than the non-smoking healthy individuals previously tested.

Investigations on the atherogenicity of polycyclic aromatic hydrocarbon (PAH) carcinogens have continued. Two major projects were completed: a revised and expanded dose-response study and a temporal response study. In both, the potent PAH carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), was the test agent and chickens were the test animal. Large, focal fibro-muscular plaques appeared in all test groups in the dose response study. These plaques were 3-12X larger than in controls. In males, but not females, plaque size was related linearly to dose. In the temporal response study, animals exposed to DMBA for as little as four weeks displayed large plaques. The number of these plaques and their average size increased with duration of exposure. Plaque formation was not reversible.

Chemical Carcinogenesis. The prevention of cancer is being pursued by identification of causative agents and by understanding of the basic cellular mechanisms which underlie the transformation of normal to malignant cells. Much of the research concerns the action of promoters and cocarcinogens which appear to play a prominent role in environmental cancer. The work described involves quantitation of the kinetics for tumor formation, development of optimal test systems in animal models for the definition and evaluation of environmental carcinogens, means of preventing cancer and an understanding of how carcinogens and tumor inhibitors act.

Studies have continued on the mechanism of haloalkane carcinogenesis. Results from the in vitro studies provide evidence of the lung microsome-dependent activation of 1,2-dichloroethane (DCE), an inducer of liver and lung tumorigenesis. Lung cytosol did not activate DCE binding. DCE binding to macromolecules was inhibited by gluthathione. The binding of DCE to DNA in the presence of lung microsomes was enhanced by phenobarbital and 3-methylcholanthrene.

Studies were completed on structure-activity in a series of tumor promoters and the relationship between tumor promoting activity and inflammatory response. Twelve components in the phorbol ester series were included, e.g., compounds in which functional changes were made in phorbol myristate acetate and compounds in which sterochemical changes were made at the 4a-hydroxyl group by epimerization. These studies indicate 1) high structural and sterochemical specificity, 2) no clearcut relationship between promoting activity and inflammatory activity on mouse skin, and 3) the primary site of interaction of the active phorbal esters to be at the plasma membrane. Membrane binding activates a series of intracellular biochemical events, some of which are related to tumor promoting activity.

It has been demonstrated that feeding raw soybean diets containing protease inhibitors decreased the incidence of breast cancer induced by X-rays in Sprague-Dawley rats. The diets employed were: a) a soybean diet containing 2% protease inhibitor, b) a Purina Rat Chow diet which contains 0.8% protease inhibitor, and c) a casein diet which contains no measurable protease inhibitor.

Rats fed the control diet (c) showed 74% tumor bearers after 11 months compared with 49% tumor bearers among rats fed the soybean diet (a). Moreover, rats fed the soybean diet had fewer tumors per rat. The spontaneous appearance of tumors in non-irradiated controls was completely prevented by the soybean diet, while in animals on the casein diet an 8% tumor incidence was observed. Fifty animals were used in each test group.

One possible mechanism for this effect is that the protease inhibitors act on the cell membrane antagonizing the formation of free oxygen radicals as shown in polymorphonuclear leukocytes by B. Goldstein, et al. Another possible mechanism is the blocking of the removal of protein repressors as demonstrated in model systems.

A protease inhibitor from snails is being purified for testing as a bladder tumor inhibitor. This material is a small polypeptide (M.W. ca 6000), which is quantitatively excreted in the urine of rats after i.v. injection.

Studies on the induction of error-prone DNA repair in E. coli. mutagenesis after UV light and many chemical carcinogens depends upon a process known as SOS repair. This repair process is error-prone and inducible, i.e., it requires de novo protein synthesis. The induction of this system requires proteolytic cleavage of repressor proteins which normally bind to DNA to keep this system turned off. When DNA is damaged by carcinogens, a signal is generated which results in repressor cleavage and the turning on of the SOS system (SOS induction).

Previously it had been found that protease inhibitors were able to inhibit mutagenesis and other manifestations of the SOS (e.g., induction of λ prophase). Data are available on a large number of different protease inhibitors which differ in their abilities to inhibit SOS induction. This gives a profile of the protease which is involved in this system and which has yet to be identified.

An investigation has been initiated on the carcinogenicity of N-nitroso derivatives of prescribed drugs. The drugs selected for the initial investigation are the anti-anxiety drug, chlordiazepoxide; the beta adrenergic blocking agent, propranolol; the ulcer healing drug, cimetidine; and the inotropic drug, amrinone. Preliminary work indicates that, depending on the reaction conditions, cimetidine reacts with nitrous acid to form two of the possible three N-nitroso derivatives. Propranolol reacts with nitrous acid readily to form N-nitrosopropranolol, which is being purified and characterized.

Previous work in this laboratory has demonstrated that squamous carcinomata of the nasal cavity could be produced in rats exposed to epichlorohydrin (ECH), dimethylcarbamoyl chloride (DMCC), and mixtures of hydrogen chloride (HCl) and formaldehyde (HCHO). A NIOSH Current Intelligence Bulletin on the carcinogenicity of ECH was based largely on work performed in this laboratory. Dimethylcarbamoyl chloride was found to be a particularly potent carcinogen since tumor yield approached 100% in rats given lifetime exposures to 1 ppm of this compound. The production of tumors in rats given lifetime exposure to the combined vapors of HCl and HCHO could represent a severe hazard since the exposure levels of 10 ppm and 15 ppm, respectively, are not far from current threshold limit values. Further investigations into the carcinogenicity of DMCC utilizing hamsters exposed to 1 ppm and rats exposed to 0.3 ppm have now been completed as has the lifetime exposure of rats to HCl plus HCHO.

The lifetime exposure of hamsters to 1 ppm DMCC produced a 51% (50/99) incidence of nasal cavity squamous carcinoma. This represents about one-half the tumor yield observed in rats given the same exposure regime. Hamsters, however, have been found to be particularly refractory to inhaled carcinogens; and this high tumor response is another indication of the extreme carcinogenic potency of DMCC.

An 8% (8/100) carcinoma yield was produced in rats given lifetime exposures to 0.3 ppm DMCC. This represents a tumor yield of less than ten percent of the tumor incidence found in rats exposed to 1 ppm DMCC and is indicative of something other than a linear dose-response pattern passing through the origin.

Radiation Carcinogenesis and Dosimetry. Radiobiological and dosimetric studies of radiation and the risks it poses are currently in progress. Problems currently under investigation include: (1) how the induction of cancer depends on the dose and the manner in which the radiation is applied, i.e., dose rate, fractionation, etc., (2) how radioactive actinides are taken up, retained and excreted in baboons and accidentally exposed humans, and (3) how the procedures and instrumentation for making low level dose estimates in tissue can be improved and made more sensitive.

Carcinogenesis is being studied in rat skin exposed to ionizing radiation, ultraviolet light or polycyclic aromatic hydrocarbons. These agents interact with the epidermal DNA in somewhat different ways. For example, measurements have been made of DNA strand break induction by ionizing radiation, pyrimidine dimer formation by ultraviolet light, and guanine adducts produced by benzo(a)pyrene, a polycyclic aromatic hydrocarbon. Each type of DNA damage was produced in proportion to the dose of respective carcinogen applied to the skin. However, skin tumors are not necessarily induced in proportion to the amount of DNA damage. For ionizing radiation, skin tumors were induced as a power of dose (exponent 2-3) while the amount of DNA strand breakage was linear with dose.

Respiratory Disease and Aerosol Physiology. A broad range of studies utilizing test aerosols are in progress. Inert monodisperse aerosols are used as *in vivo* probes to study air space sizes in the bronchial and alveolar regions of human and animal lungs, and to characterize deposition patterns and efficiencies within these airways. In addition, specific aerosols are being used to determine their *in vivo* effects on respiratory mechanics and particle clearance from the bronchial and alveolar airways. Those include sulfuric acid, ammonium bisulfate, and ferric sulfite, while aerosols such as magnetite, coal, and mineral fibers are used in the studies on alveolar clearance. Particle retention measurements in these latter studies are made with both external γ-radiation detectors and a gradient magnetometer.

A series of studies are being completed of aerosol deposition in multiple replicate hollow human airways casts extending from the larynx to the 3mm diameter bronchi. This study involves a comparative investigation of the intrabronchial pattern of aerosol deposition under steady and simulated inspiratory flow. Since laryngeal size changes *in vivo* with inspiratory flow, a variable opening "larynx" was designed and constructed for use in this study.

Airflow measurements are being performed using probes placed within the airways of the hollow case in an attempt to relate differences in overall and intrabronchial deposition patterns to differences in airflow patterns.

A microscopic analysis of deposition along a major bronchial bifurcation is being performed. The results will provide a basis for more detailed and refined deposition models for toxicant and radionuclide dosimetry. Preliminary observations indicate an enhancement of deposition along the carinal edge.

Stroboscopic techniques will be employed to determine the beat frequency of tracheal cilia. Incorporation of radioactive precursors into total pulmonary glycoprotein and airway glycoprotein will be qualified as measures of mucus synthesis and secretion, respectively. Collection of tracheobronchial secretions at the level of the larynx will permit quantitation of total mucus transport and examination of mucus rheological behavior using capillary viscometric techniques. The effects of sulfur oxides, particularly H_2SO_4 aerosol, on the mucociliary system will be examined with these test protocols.

Epidemiology, Biostatistics and Biomathematics. Research in biomathematics, biostatistics and epidemiology includes respectively: 1) the theoretical formulation of mathematical models for extrapolation to low dose levels and for evaluating the temporal pattern of effects when a general population sustains chronic environmental exposure to toxicants; 2) the development of effective methods for the design of experiments and analysis of environmental health data; and 3) the application of these methods to epidemiological studies of the distribution and content of findings on environmental toxicants and the quantitative assessment of health effects and human environmental health hazards. In addition, the Biomathematics and Biostatistics Laboratories provide important teaching and technical support for many other research programs in the Institute.

An epidemiologic study of minimal breast cancer is in its third year. Data are being collected for an estimated 1,000 valid cases of breast cancer and approximately 2,700 controls selected from women screened at the Guttman Institute through October 1979. The first phase of the analysis will be a determination of relative risk estimates from the comparison of all cases to controls, which should result in estimates within the range found at other screening centers. Data collection and editing for this phase is about 80% completed. Subsequent analysis will require the results of pathologist review of slides and hospital reports which will characterize, among other things, the level of invasiveness (minimal vs. clinical) and histologic type. These criteria, along with a measure of tumor aggressiveness (prevalent vs. incident disease) will be used to assess risk factors associated with minimal as opposed to clinical disease and prevalent as opposed to incident disease. The pathology review, which features concensus between two independent reviews of the same material by different teams of pathologists, is about 25% completed. Current sample size projections indicate that 350 minimal cases will be available for comparison to 650 clinical cases.

The association between hair dye use and breast cancer continues under study. Constituents of hair dyes shown to be carcinogenic in animals are known to be absorbed through the skin and to enter the systematic

circulation. A history of hair dye use was obtained from a series of breast cancer patients and a comparable group of women without breast cancer. The analyses, which examined the duration, frequency and temporary pattern of hair dye use, showed an exposure response relationship between breast cancer and integral hair dye use. A temporal analysis indicated the relationship was primarily for integral hair dye use ten or more years before cancer diagnosis, which is consistent with expectations regarding a tumor induction-latent period. There was also evidence of an interaction between hair dye use and other risk factors for breast cancer. A larger confirmatory study has been planned.

Environmental Pollution and Ecology. Studies in environmental pollution and ecology address the effect man's activities have on his air, land and water environments. All studies are integrated to the extent that the pollutants of air and water (heavy metals, organics, radionuclides, etc.) cycle in a dynamic manner in the environment. Present research on the sources, sinks and distribution of specific contaminants in the Hudson estuary and the coastal marine environment, for example, rely heavily upon simultaneous studies of these pollutants in urban aerosols, since atmospheric transport to aquatic systems may represent a significant input source. Similarly, studies of particular pollutants in air and water contribute to an understanding of the cycling of other materials since the kinetics of sorption, solution, ion exchange and biomagnification is similar in many classes of compounds. Air and water pollution studies are directed toward an increased understanding of the ways pollutants behave in natural systems and work their way back to man.

The distribution of PCB in organisms throughout the tidal Hudson River is being studied. Seven stations have been established covering a 165-mile stretch of the Hudson estuary from lower New York Bay to Albany. Samples of ichthyoplankton, macrozooplankton and microzooplankton are collected at each station for subsequent extraction and analysis for PCB content. Sampling runs are made thrice yearly, in May, August, and October. Samples are sorted by hand so that, whenever possible, PCB analyses can be made on a species-by-species basis.

The data show a pronounced gradient of PCB contamination from upstream (Albany) decreasing toward New York Bay. Zooplankton from different taxa show different levels of PCB contamination, with juvenile fishes having less PCB than planktonic crustacea. The data are used to illustrate that all trophic levels of the Hudson food chain are contaminated with PCB and that contamination of the biota follows a trend similar to the PCB found in the River sediments.

Currently these studies are being expanded to include a more comprehensive sampling effort in the New York Harbor/Raritan Bay region, and a more in-depth analysis of food species.

PCB uptake is rapid in all species tested. The crustaceans accumulate PCB to greater levels than fish in 48-hour exposures, although rates of uptake are more rapid in fish during the first 12 hours of exposure. Uptake may occur directly from the water, or via ingestion of contaminated

food. Uptake from the water is facilitated by the presence of suspended particulate material.

Currently studies are underway to assess physiological effects of PCB on fishes and amphipods. Aroclor 1254 has been shown to have a direct effect on amphipod respiration; effects on long-term reproduction and cellular adenylate charge ratio are being investigated. Fish studies are concentrating on the distribution of PCB in various tissues after uptake, and on determining the complement of PCB isomers in the organism as opposed to that in the source material. The impact of low-level PCB contamination on Gammarus population dynamics is being evaluated in long-term experiments.

The results of these and other studies aimed at evaluating the environmental behavior of PCB will be considered in current efforts to devise schemes appropriate for "cleaning up" bodies of water contaminated with PCB.

Massachusetts Institute of Technology

The Center's activities at the Massachusetts Institute of Technology are performed under the aegis of Harvard University - MIT Division of Health Sciences and Technology and MIT Energy Laboratory, and involve participants from the MIT Departments of Nutrition and Food Science, Chemical Engineering, Mechanical Engineering, Biology, Chemistry, Material Sciences and Engineering, Aeronautics and Astronautics, and Harvard Medical School.

Combustion engineers are investigating the formation of organic particulates and polycyclic aromatic hydrocarbon compounds in a range of combustors from small-scale laboratory flames to semi-pilot-scale equipment. The research involves collection and detailed characterization of soots and other emissions formed by combustion of vaporizable and non-vaporizable fuels, pulverized coals, coal- and shale-derived liquids, and residual oils, with special emphasis on fuels with low hydrogen-to-carbon ratios. The work will also include collection and analysis of selected field samples from pilot and commercial-scale combustion equipment such as pulverized-coal-fired utility boilers, domestic oil burners, and fluidized bed coal combustors.

Chemical analyses of the emissions products from the various combustors will be used to identify the specific organic compounds present. Chemists are using advanced instrumentation and gas chromatographic-mass spectrometry techniques to quantify polycyclic aromatic compounds.

Toxicologists are assessing biological activity of the particulate samples and individual organic compounds that have been identified and isolated in the combustion experiments. Specifically, they are using both human cell and bacterial forward mutation assays to determine mutagenic activity of those materials, and will further test those indicating positive responses for carcinogenicity. The biological assays use protocols chosen to reflect as closely as possible the responses expected from actual tissues. Other toxicologists will assess

the mutagenic effects of emissions materials on a microscopic level by studying the interactions between the molecules of the human body and those of specific test compounds representative of fossil fuels emissions. These studies should indicate how molecular structures interact to form or break chemical bonds and in the process cause alterations in the human genetic code.

Basic Studies of Organic Particulates from Vaporizable Fuel Combustion. Research is currently being carried out on two basic types of flame: a reactor in which fluid mechanically-induced mixing is rapid compared with the chemical reactions taking place (the "well-stirred reactor") so that only chemistry controlled variables are important, and in laminar flow systems where turbulent mixing is eliminated but where concentration gradients set up by the chemical reactions taking place exist and are important. It is expected that the effect of combustion conditions can be approximately described by considering practical equipment to produce a quantity and composition of organic particulates that is a combination of these basic extremes, the proportions depending on the specific design of the equipment.

In the course of this work, several technical problems were identified. First, the flame was not flat enough to allow internal sampling. This was attributed to non-homogeneity of the temperature of the burner ceramic plug, and to molecular diffusion in the flame itself. Second, the exhaust gases are not totally mixed with the surrounding nitrogen shield at the sampling point. Consequently, the exact fraction of flame gases collected is not known. A new burner and sampling system, providing better flame stability and the total collection of the exhaust gases, have been designed and built to overcome this problem. The exhausts of a toluene/oxygen enriched air flame were collected with this new equipment. More than fifty compounds have been separated, by glass capillary column on the gas chromatograph, from the methylene-chloride extract of collected particulates. They are identified by gas chromatography-mass spectrometry and their mutagenic activity is being assessed.

Basic Studies of Organic Particulates from Coal and Heavy Liquid Fuel Combustion. The formation of organic particulates and polycyclic aromatic hydrocarbons (PAH) is being studied under well-defined laboratory conditions simulating the temperature and oxidation histories expected in practical combustors. Emphasis is placed on pulverized coals, coal-derived liquids and residual oils with high carbon/hydrogen ratios. The conditions of primary interest are those in which the particles or droplets form diffusion flames, a condition known to favor soot formation. The experiments are designed to systematically cover temperature equivalence ratios and residence times in order to identify the regimes in which soot and extractable organic matter are formed. Selected screened conditions will be used for more exhaustive chemical analysis and toxicological testing.

The research effort during the period was directed to development of techniques and procedures for the collection of representative PAH samples from within turbulent flames and from the freeboard of fluidized

coal combustors. The sampling problem is due to several factors: (a) The samples contain several phases--partially burned combustible solids and liquid fuel droplets, fly ash, soot, gas and condensible vapors. (b) These phases are difficult to separate. (c) It is difficult to ensure that each element of the sample is quenched efficiently and that condensation occurs only in the desired place in the sampling train.

In preliminary experiments, a water-cooled sampling probe equipped with a sintered bronze filter was used for "in-flame" measurements and PAH compounds found absorbed on soot were analyzed for their composition. Following these preliminary experiments, two sampling systems were designed and constructed; the first of these for turbulent flames studies and the second for use in fluidized combustion experiments. Also, an Acurex large volume organic module/sorbent trap was acquired, mainly to collect samples from the exhaust gases of both types of combustion system.

Experience in experimental technique and probe/sampling system design was obtained by using the "in-flame" sampling probe and train to determine the solids concentration and PAH content of sampled solids. The PAH content of the condensible organic vapors present in the exhaust gases was also determined, from flames obtained with a Californian high-nitrogen content No. 6 fuel oil. Staged combustion techniques offer great promise for controlling NO_x emissions from the combustion of high-nitrogen content fuels. However, the staged-combustion conditions are conducive to the formation of high concentrations of soot and PAH in the fuel-rich first stage of the flame.

The second sampling train is currently being fitted for experiments on the 2' x 2' pilot plant fluidized combustor. The special feature of this sampling system is that it separates large particles from smaller particulates and the gas by means of a small cyclone precipitator placed at the tip of the probe. Preparations have also been made for the design and development of a large volume sampler which would obviate the presently necessary long periods of sampling (approx. 2 hours per sample) when sampling in low PAH concentration flue-gases.

Analysis of Polycyclic Aromatic Compounds. The purpose of this project is to provide analyses of organic polycyclic aromatic compounds and to develop or improve analytical methodology for samples generated by the combustion engineers. During the period covered by this report, the major effort was channeled towards PAH analysis. In addition, much time was spent on instructing the engineers about sample preparation methods and how to avoid contamination. In effect, the technical interaction between the various engineering groups and the chemists has been developed and strengthened.

The organic compounds produced by the combustion of different types of fossil fuels are qualitatively and quantitatively analyzed by gas chromatographic-mass spectrometry. The compounds in these combustion effluents include polycyclic aromatic hydrocarbons (PAH) and poly-heterocyclics containing sulfur and nitrogen. Although existing analytical

methodology for these compounds is effective, there are certain limitations which need to be addressed; for example, the electron impact mass spectra of isomeric PAH are almost identical. Therefore, in addition to analyses using current techniques, more advanced analytical methods are being developed for these compounds. Preliminary experiments have indicated that charge exchange-chemical ionization mass spectrometry (using 5 percent methane in argon as the reagent gas) can differentiate PAH isomers. This technique will be combined with high resolution gas chromatography using both non-polar and liquid-crystal liquid phases to provide still more effective methods for the analysis of complex PAH mixtures. Analysis of the sulfur and nitrogen containing poly-heterocyclics will also be performed.

Experiments with various temperatures and oxygen contents for two coal types were carried out. The combustion of coal at 1250°K and 0 percent oxygen produced the highest amount of PAH. The toluene extract of the combustion products from the Montana lignite and the Pittsburgh No. 8 bituminous coals produced 28 identifiable PAHs.

Monitoring Combustion Products by Laser Spectroscopy. Laser-induced fluorescence excitation spectroscopy (LIFES) is being evaluated as an *in situ*, real-time diagnostic for polynuclear aromatic hydrocarbons (PAH) formed in combustion sources. If such a method can be successfully demonstrated, then assessment of the potential PAH burden on the environment, as a result of increased utilization of coal and other "dirty" fossil fuels, will be made considerably more precise and convenient, and national health policies and decisions related to the allowable use of these fuels can be made with greater confidence.

Initially, a search was carried out for available literature data on vapor-phase fluorescence excitation and emission spectra of individual C_{10} C_{26} PAH species. The information is sufficient to permit the design of an experimental protocol, but is inadequate to serve as the required data base on excitation spectra for pure species. Accordingly, the initial experimental measurements are directed at establishing excitation profiles and quantitative response factors for the species of interest.

Fluorescence excitation and emission spectra have been obtained for four representative PAH species, viz.: perylene, anthracene, pyrene, and fluoranthene, the latter two species in an ethylene-oxygen flame as well as in a static cell. Spectra of individual species can be distinguished on the basis of both their excitation and their emission profiles.

Single Cell Assays for Gene Locus Mutation. The role of this project in the Center is to provide bioanalytical support. Development of a convenient assay for forward mutation in *Salmonella typhimurium* and its application in studies of mutation by PAH and soot represent the principal contribution of the project to date. In addition, a laboratory facility has been established which can respond to the needs of colleagues by rapid and accurate bacterial mutation assay on as many as 50 samples per week.

Bacterial mutation assays presently play a major role in toxicological screening programs and in biochemical studies probing the mechanism of mutation. The system most commonly used today is the Ames Salmonella test, in which reversion to histidine prototrophy is used as a genetic marker (McCann and Ames, 1976). The Ames assay is presently used by over 50 major drug and chemical companies to test their products for mutagenicity. Also, hundreds of laboratories routinely use this system as an investigative tool to study chemical mutagens and the mutagenic process.

The relative sensitivity of the 8AG, FU, and ACA systems will be further tested by comparing the mutation induced with a variety of mutagens of both the frameshift and base-pair classes, including some direct-acting compounds, and some which require metabolic activation.

The mutagenic activity of the PAH of sulfur-containing soot, nitrogen-containing soot, furnace black, and kerosene soot were measured in a Salmonella typhimurium forward mutation assay. Each of these samples was found to be mutagenic at concentrations of 20-50 μg/ml culture medium in a two-hour exposure (Aroclor-preinduced rat liver post-mitochondrial supernatant). Initial slopes of the concentration dependence of induced mutation show the sulfur-containing soot to have 10 percent of the activity of pure benzo(a)pyrene; the nitrogen-containing soot, 10 percent; furnace black, 13 percent; and kerosene soot, 17 percent.

Several other soot extracts are being evaluated with regard to the mutagenicity of their individual components. Of particular importance will be a complete characterization of several diesel soots. If additivity of mutagenic activity is indeed a general phenomenon among soots, enough data to establish this concept should be available by the end of 1979.

In the first year of this project attention was focused on the aza-derivative of phenanthrene benzo(f)quinoline (B(f)Q). This compound has been identified in suspended particulate matter from New York City air and in lake sediments. It has recently been shown to be mutagenic to Salmonella typhimurium in the forward mutation assay.

Initially, fluorescence spectroscopy was to be used to monitor binding of B(f)Q to DNA. It was a logical choice for several reasons: (a) the radioactively labeled compound was not available; (b) DNA does not fluoresce at neutral pH; (c) B(f)Q has a strong fluorescence spectrum, well documented in chemical literature. However, the shortcomings of this technique were soon discovered. For one thing, the binding could not be quantitated. Secondly, in order to detect low binding levels the spectrophotometer was set on a very high sensitivity which, in turn, created problems because of solvent impurities and the high viscosity of DNA solutions. In addition, if no fluorescence was detected, it would not be known whether this reflected lack of binding to DNA or quenching of B(f)Q fluorescence due to the electronic changes in its environment, or simply lack of fluorescence in the metabolites of B(f)Q. Consequently, labeled B(f)Q was synthesized by a route which led to incorporation of tritium atom at the C-9 position of the molecule.

Bacteriophage as a Model System for Studying the Molecular Toxicology of Heterocycles from Coal Combustion. This project involves the use of bacterial viruses containing double stranded DNA, as model systems for examining the interactions of aza-heterocycles with DNA in its organized forms. The major focus has been on the effects of aza-heterocycles in the presence of sunlight, dye-sensitized photoinactivation. During the past year it has been shown conclusively that with acridines as the sensitizer, the acridines are taken up by DNA, but the major targets of damage are specific proteins which are probably bound to the DNA. The mechanism presumably involves a singlet oxygen or other transient intermediate, which is generated in very close proximity to the sensitive amino acids of the target proteins. As a result of the damage to two species of proteins in the virus particle, the inactivated particles are unable to inject their DNA into the host cell.

A second major line of investigation has been initiated in the effects of acridines in the dark. Though long known to be toxic, the mechanisms have never been directly elucidated. A study on the effects of acridines on inhibiting the intracellular assembly of virus particles has shown that acridines interfere with this process of assembly. Most of the steps in P22 assembly proceed in vitro, in mixtures of extracts of mutant infected cells. Using this in vitro system it has been shown that a number of the acridines, but not all, inhibit the DNA packaging process, and not later steps in viral maturation.

Biological Impact of Diesel Particulates. The focus in this project is on effects of particulate emissions from diesel engines. As awareness of the potential mutagenic activity of combustion particulates expands, these emissions have been of growing concern because of the extensive and increasing use of diesel engines in transportation.

In the past, diesel particulate studies were generally organized to screen engines and vehicles through a "typical" driving cycle to examine the characteristic biological activity of "typical" soot extracts from these engines. These programs do not, however, yield insight into either the physical processes controlling formation, burn-up and change of composition of the soot in the system; nor do they yield a control strategy. In this program project members seek to combine the facilities at the MIT Sloan Automotive Laboratory with new techniques developed under the NIEHS grants to explore the fundamental issues that characterize the combustion of a diesel engine. The feasibility study rests on two tasks: 1) To design a basic facility for collecting and analyzing the exhaust particulate matter from a small diesel engine; 2) To develop the basic technology for acquiring and analyzing the soot samples from a diesel engine.

Development of Lung Perfusion Model for Toxicologic Studies. This study is aimed at developing a well-characterized model of isolated lung perfusion to be used for assessing the impact of toxic and carcinogenic compounds on the lung. The metabolic integrity of these preparations cannot be said to function under reproducible physiological conditions. Experience with perfusions of isolated livers, kidneys and testes will

be used to select the optimal conditions for setting up a reliable model. The performance of lungs ventilated by negative (artificial thorax) and positive (direct ventilation) pressure will be compared. In addition, strict metabolic parameters assessing the functional integrity of the organ will be defined. The model will also be characterized by its capacity to metabolize or excrete selected toxic substrates. Lastly, the binding to lung DNA of model carcinogenic compounds will be quantitated.

University of Cincinnati

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. Lead remains a focus of research. Cadmium also continues to be a metal of major interest to many members of the department. There are projects addressing problems of mercury and aluminum as well.

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 being studied. Neurobehavioral effects of lead exposure are further considered in the section on Neurobehavioral Studies. That section also summarizes research on the effects of lead and cadmium on synaptic transmission.

The lead-exposed rat was used in further work on the mechanism of renal aminolevulinic acid (ALA) excretion. While earlier work had not revealed any evidence for tubular secretion of this compound in rabbits, there is now some suggestion for its active secretion by the rat kidney. The significance of possible secretion in explaining the increased plasma and urine levels of ALA following lead exposure remains to be elucidated.

As part of an assessment of the lead exposure in a population of 24 brass foundry workers, activation of erythrocyte ALA dehydrogenase (ALAD) by Zn *in vitro* was studied. Such a reactivation had previously been noted in rats and in an isolated lead-exposed patient. An intriguing observation was that the addition of zinc elevated ALAD activity above that seen in the red cells of control subjects. Apart from the clinical or toxicological significance of lowered ALAD levels, it is interesting to note that zinc supplementation during chelation therapy with EDTA in one patient resulted in an increase in ALAD levels.

The effects of Cd on renal function continues to be a major focus of the toxic metals. One project attempted to explain in molecular terms the tubular proteinuria typical of Cd poisoning. This work was based on the finding that ionic charge is a major determinant of protein reabsorption, and that the first step in this reabsorption (the binding of the protein to the brush border membrane), as measured *in vitro*, is greatly increased by various divalent metal ions, such as zinc and calcium. *In vitro*, Cd ion at certain concentrations was found to antagonize the action of Ca. Calcium treatment *in vivo* increased the Cd content of the brush border

membrane to the level at which in vitro experiments had shown inhibition of protein binding at the brush border membrane.

Additional work was also 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. The 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.

Further progress has been made in attempts to elucidate the mechanism whereby inorganic mercury produces acute renal failure in rats. The well-documented ability of the kidney rapidly to accumulate mercury may be related to the observation that mercury is not only filtered and reabsorbed in the tubule, but can also be extracted directly from peritublar blood. The onset of acute renal failure, as previously reported, is greatly modified by treatment of the exposed animal with the sulfhydryl reagent dithiothreitol. The mechanism of action of this compound remains uncertain. Thus, no difference in the tissue distribution of injected mercury, or in the intrarenal partition of Hg could be observed between control and treated animals.

Pulmonary and Inhalation Toxicology. A study was completed on the effects of aluminum sulfate exposure on pulmonary function in rats. The 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 study was undertaken of the influence of particulate carbon on the effects of nitrogen dioxide during a 6-month exposure of adult rats for 8 hours per day at a concentration of 2.5 ppm. After two months, slight increases in lung and body weight were seen in animals exposed to both carbon particles and nitrogen dioxide. No changes could be observed in residual volume, vital capacity, alveolar volume, carbon monoxide transfer factor, airway resistance or lung compliance. Preliminary results suggest that the effects of chronic exposure to carbon dust and nitrogen

dioxide may be different from the additive effects of exposure to the two individual substances. As part of this work, a carbon particle generator was developed in the laboratories of the Environmental Hygiene Division which can function continually for 8 hours on one charge of approximately 100 grams of charcoal.

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.

Human Studies: Clinical and Epidemiological. The 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 ration (SMR) for all causes of death in this group was shown to be 0.69 with 32 deaths observed and 46.41 expected. For categories of malignant neoplasm and circulatory diseases, SMR's 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. 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, and the age of the patient, the duration of the disease, or the degree of airway obstruction as measured by pulmonary function tests.

A study was conducted on a group of workers exposed to isocyanates in the polyurethane foam process. This study included a detailed history, pulmonary function tests, blood studies and industrial hygiene measurements. Abnormal pulmonary function tests indicating airway obstructive disease

were noted in a large proportion of the exposed individuals. The investigation provides an epidemiologic method for indentifying cases of occupational asthma in industry. The population can be further studied with bronchial provocation testing in order to confirm the diagnosis. Such laboratory studies should aid in validating the accuracy of the methods used for epidemiologic studies.

Considerable attention was devoted to the development of more quantitative methods for performing bronchial challenge studies with a variety of occupational allergens in man. These studies were carried out in the General Research Center and have demonstrated definite dose-response relationships to various inhalation challenges. The study suggests that use of mecholyl as a screening test for identifying susceptible and sensitive workers, although helpful in some circumstances may not be practical since the absence of a positive test does not exclude sensitization to, for instance, toluene diisocyanate. Another study involved a detailed medical examination and testing of pulmonary function in workers exposed to polymer fumes. This study has so far shown that even chronic exposure to polytetrafluoroethylene fumes and repeated attacks of polymer fume fever do not result in chronic pulmonary disease. The mechanism of this illness remains unknown.

Several studies have been conducted to determine the effect of toxic agents on male sexual function which utilizes various parameters of semen analysis. The agents under study are carbon disulfide, toluene diamine and estrogens. The work with carbon disulfide involves workers at a viscose rayon plant. Exhaustive statistical comparisons of the results obtained from various laboratory measurements have provided no evidence for significant differences between control groups and the group exposed to carbon disulfide at the present level of exposure. Another investigation of possible reproductive toxicity in male workers involved the investigation of an alleged increase in spontaneous abortions of wives of male workers employed in manufacture of toluene diamine. The field work for this study has been completed, and results are at present being analyzed statistically.

Work is continuing on the definition of better indices of lead exposure and of their biological significance. The initial phases of this study, now completed, established the relative ability with which several indices of exposure to lead in a secondary smelter may be correlated with health effects. Deficiencies in renal function were observed in lead workers whose renal status as reflected by blood urea nitrogen or serum creatinine falls within the normal clinical range; current studies involve more detailed renal studies on such workers.

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. The members of this group frequently provide collaborative support for many projects which require sampling, analysis, the design and operation of inhalation chambers, and environmental survey work.

Development was undertaken 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. In addition, the analytical laboratory carried out numerous trace metal analyses both for investigators in the department, as well as for other groups at the Medical Center.

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 biomass was highest in the fertilized plots and lowest in control plots, there was 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 hexachlorobicyclohepadiene. Some samples proved moderately to severely toxic to cultured mammalian cells. There was also evidence for some mutagenic activity.

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.

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 liquid 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.

Cocultivation of an indicator cell with the non-irradiated hamster embryo cell permitted further testing of the two stage hypothesis of

cancer with proximate carcinogens. Initiation is an irreversible change in the genone of sensitive cells and this step could be quantitated by counting ouabain- or thioguanine-resistant mutations resulting from subsequent treatment with an ultimate carcinogen. Promotion could then be defined as the increase in the number of mutants following treatment of initiated cells by classical tumor promoters. Several classes of agents known to be promoters in vivo have been found to enhance mutation frequencies induced by other mutagens. Criteria which characterize tumor promotion in vivo have also been found to apply to mutagenesis enhancement in vitro, in reference, for instance, to the time of addition of, and period of exposure to the promoter. In common with mouse skin epidermal cells in vitro and in vivo, V79 test cells have also been found to respond to phorbol esters with a large increase in ornithine decarboxylase activity and a significant increase in putrescine content.

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.

Studies are proceeding on the chemical aspects of carcinogenesis. Thus, the non-bonding interactions of polycyclic aromatic hydrocarbons with DNA were followed by fluorescence techniques. Carcinogenic and non-carcinogenic hydrocarbons were compared in terms of fluorescence life times, fluorescence quantum yields, intersystem crossing quantum yields and internal quantum yields of compounds such as benzo(a)pyrene, benzo(e)-pyrene, chrysene and benz(a)anthracene. Other studies have been initiated on the effects of dietary-inducing agents and inhibitors on pulmonary cytochrome P-450 and related enzyme activities. The group of compounds of primary interest in this study are the N-nitrosamines and their precursors such as secondary and tertiary amines. The pulmonary metabolism of N-nitrosamines is being analyzed with isolated lung microsomes, as well as with the isolated perfused rat lung. These compounds are oxidized by a cytochrome P-450 mediated mixed function oxidase; the mutagenic products can give rise to lung adenomas in experimental animals.

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.

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 had 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.

Mount Sinai School of Medicine

Polybrominated Biphenyls. Neurological symptoms were the earliest and most prominent symptoms in Michigan residents exposed to PBB as compared to a non-PBB exposed population in Wisconsin. In Michigan (particularly among males) those who exhibited the most marked symptoms tended to show diminished performance as assessed by special tests although population differences in performance were not as marked. Subjects exhibiting the most prominent neurological symptoms were found to have had significantly higher intake of home-produced foodstuffs (particularly combination of foodstuffs such as beef, pork and butter) in 1972-1974 and store-bought products in 1975-1976. Low indices of performance were also correlated with intake of home-produced foodstuffs, particularly during the years 1972-1974 and store-bought products during the years 1975-1976. From 1972 to 1976 Michigan residents made significant changes in their consumption patterns of products suspected to be contaminated with PBB, as compared to those of Wisconsin residents.

Michigan males and females with neurological symptoms were not found to be significantly different in any of seventeen different blood chemistry tests (SMA's and CEA). In Michigan males, SGPT and SGOT levels were negatively correlated with performance. A highly negative correlation was also observed for CEA, cholesterol, creatinine and sodium levels. Calcium, protein and bilirubin levels exhibited a positive correlation with performance. In Michigan females, a significant negative correlation between CEA, cholesterol, creatinine, uric acid and LDH was also observed. In Wisconsin males and females no similar trends were observed.

Serum PBB levels were not found to be significantly different in Michigan males and females exhibiting the most prominent neurologic symptoms. Serum PBB levels were negatively correlated with performance test scores, particularly in males in older age groups.

Asbestos Workers. Considerable progress has been made in the collection, preparation and analysis of data from the five cohorts of asbestos workers under current investigation. Data processing has progressed sufficiently to allow the presentation of descriptive statistics and

preliminary analyses of information collected in the field survey of shipyard workers in Baltimore.

Collection of data from three additional cohorts (members of Locals 12 and 32 of the International Association of Heat and Frost Insulators and Asbestos Workers and former employees of Union Asbestos and Rubber Company) is continuing. Seven field surveys have been undertaken in Paterson, New Jersey to update medical data and to obtain (by interview) detailed information on the economic and social impact of disability on cohort members and their experience with workers' compensation, Social Security and other sources of support and compensation. Previous medical records for each of those currently examined are being reviewed, abstracted, and prepared for computer entry.

Interviews with over one hundred members of these cohorts have been keypunched and are now undergoing further preparation for proofing and statistical analysis. As with the Key Highway workers, releases are being collected from each person for records from the Social Security Administration. Data on disability insurance applications, Medicare costs and usage and summary earnings records since 1951 are being processed at this time.

Surviving next-of-kin of members of the UNARCO cohort known to have died of asbestos-associated diseases from 1967 through 1976 are being tracked. Since family contacts of some deceased UNARCO cohort members are currently under clinical observation in the Paterson surveys, it is planned to conduct personal interviews concerning the survivors' experience with workers compensation and other issues of interest at the same time.

Data has been obtained on workers' compensation claims and awards paid to survivors of members of the International Association of Heat and Frost Insulators and Asbestos Workers in Pennsylvania and New Jersey known to have died of an asbestos-associated disease, 1967-1976.

In New Jersey, forty-four members of the Union's three locals in Newark, Trenton, and Atlantic City had died of an asbestos-associated disease. Claims for workers' compensation benefits were made prior to or after death in twenty-five cases. Partial permanent, permanent total and/or dependency (survivorship) benefits were awarded in nineteen cases. Claims were dismissed in four cases and two death claims (in 1972 and 1974) are pending.

National Survey Asbestos-Associated Deaths. Considerable time was spent preparing for interviews with survivors of the National Survey cohort. Over 1,100 deaths from asbestos-associated disease have occurred in this cohort from 1967 to 1976 and procedures are being developed so that maximum information will be obtained from all knowledgeable sources.

Information on ninety-nine deaths from mesothelioma have been abstracted from existing Mount Sinai files. The last address of the surviving spouse or other kin is available in most files. About one-third have no phone listing in the local directory and several have unlisted phone numbers.

Based on experience thus far, it is hoped that survivors can be found in more than half of the National Survey deaths. Of the sixty people contacted so far, only one was hostile and refused cooperation with the study. The rest were very cooperative and hopeful that they can contribute to a study that will help others.

The International President of the International Association of Heat and Frost Insulators and Asbestos Workers, AFL-CIO, has agreed to assist in efforts to contact surviving relatives. A letter will be sent to all local union business agents asking for their assistance in locating survivors with whom we had not made contact. It was found to be quite common for a son of a deceased asbestos worker to be a current member of the same local union. It is also common for other relatives to be active union members, so that these sources can be tapped even if the business agent is unable to help.

Quincy Shipyard Field Survey. A field survey of four hundred shipyard workers employed by General Dynamics Corporation in Quincy, Massachusetts from November 2-5, 1979 is being planned. The disability compensation questionnaire and medical protocol will be similar to those used at Key Highway shipyard. The data from this survey, coupled with those available from Baltimore, will allow a more complete understanding of the disabling effects of asbestos-associated disease among shipyard workers.

Disability Compensation and Related Issues at Key Highway Shipyard. Asbestos-associated disease was first suggested to be an important problem of shipyard workers by European investigators in 1968. The problem is potentially of even greater significance in the United States than elsewhere as our country undertook much shipbuilding and ship repairing for the free world during World War II.

It was reported recently that approximately 4.5 million men and women were employed in the United States naval and civilian shipyards during World War II. It is known that widespread asbestos exposure occurred during these years and an integrated analysis of the limited information available on dust levels suggests that overall time-weighted averages ranged between 4 and 12 fibers/ml. With few industrial hygiene precautions taken, exposure was not limited to those in the typical asbestos and insulating trades. Working in confined, enclosed areas, many other shipyard trades were similarly exposed to asbestos.

Results from a clinical field survey of 1,000 shipyard workers in Groton, Connecticut, undertaken in 1975 and 1976, were also reported. Overall, approximately half of the workers examined showed x-ray evidence of pulmonary and/or pleural changes of the type regularly seen following direct or indirect occupational exposure to asbestos. One or another abnormality was present in 274 of 636 workers with less than twenty years from onset of exposure and among 185 of 364 workers twenty or more years since onset.

No trade at the Groton yard was immune to such pulmonary and pleural changes. Duration from onset of exposure was important, with 13.9% of those 0-9 years from onset with abnormalities, 41.7% of those 10-19 years, 52.2% among those 20-29 years, and 61.0% for those thirty or more years from onset.

Although pulmonary and pleural abnormalities, visible by x-ray examination, indicate prior significant exposure to asbestos, these changes alone do not indicate degrees of impairment or disability. Limited x-ray changes, however, often precede lung cancer, mesothelioma and extensive asbestosis. A survey to determine extent of disability among shipyard workers at Key Highway was undertaken in conjunction with the standard Mount Sinai protocol for investigation of asbestos-associated disease.

Asbestosis Among Household Contacts of Asbestos Factory Workers. The environmental burden of asbestos pollution is a recent phenomenon which has grown with the rapid expansion of asbestos-utilizing industries. The health consequences of poorly-controlled occupational exposures to chemicals and dusts now found in the general environment have been known in many instances for well over 100 years. Reports of overt disease (usually seen only with occupational exposure) among non-occupationally exposed individuals have frequently been considered medical curiosities when they appeared in the medical literature. However, the full extent of the health risks due to non-occupational exposure to toxic agents are not known, for it is uncommon to inquire into the neighborhood residence history or occupation and exposures of a patient's household contacts when investigating symptoms of a disease. The effects of such exposures may be mild or subclinical manifestations which are only contributory to a current health problem and their role goes unrecognized.

In 1976, a systematic investigation of one such non-occupational exposure to asbestos dust was reported. The group studied consisted of household contacts of workers in an asbestos factory manufacturing amosite asbestos insulation materials between 1941 and 1954. None of those reported had personal occupational exposure to asbestos. Yet 35% had asbestos-associated radiographic abnormalities. A source of home contamination in individual exposure was postulated as resulting from dust adhering to shoes, hair, and workclothes brought home for laundering. Changerooms and company-laundered coveralls were not available at this plant. Four pleural mesotheliomas among the family contacts of the 1,664 workers who were employed at some time by the factory have been identified. Since that time, one additional pleural mesothelioma death has occurred, raising the total mesothelioma deaths to date among the group under observation to five.

Short-Term Asbestos Work Exposure and Long-Term Observation. There is considerable interest in dose response relationships involving exposure to carcinogenic agents. However, a major difficulty in establishing these relationships is that there usually is a lengthy latent or induction period between the exposure and the subsequent overt emergence of cancer.

The mortality experience of a group of Paterson, New Jersey, amosite asbestos factory workers from the onset of work 1941-1945, through 30 years thereafter, was reported previously. Some of these men had a very limited duration of direct asbestos exposure.

It was found that work exposure to amosite asbestos for as short a period as one month showed a clear excess risk of cancer. With longer direct exposure (i.e., 2 months, 3 months, 6 months, etc.) the cancer risk became greater. With very brief direct exposure, cancer risk was found increased only after 25 years. Longer employment resulted in excess cancer after shorter post-exposure observation periods.

The observation period has been extended to 35 years after onset of work, to locate several men previously lost to follow-up, and to thoroughly review the information recorded for each man. Mortality experience are being determined for persons of the same age group at different 10 year periods of time after onset of work. Of particular interest will be the findings in a short time after onset for men in their forties and fifties.

Harvard University

Environmental Lung Cancer. This investigation is designed to examine factors which influence the induction of experimental lung cancer in hamsters exposed to alpha radiation and chemical carcinogens. Recent results show a significant synergistic interaction between low doses of alpha radiation and benzapyrene administered 4 months later. Most of this effect can be ascribed, however, to a potentiating effect of subsequent saline instillations on alpha radiation carcinogenesis. Recent results indicate that exposure to isotonic saline under these conditions leads to a transient wave of cell proliferation amongst the bronchiolar epithelial cells, the site of the malignant tumors. A working hypothesis that this stimulus to cell proliferation acts to facilitate expression of the initial radiation damage.

Mutagenesis and Carcinogenesis in Mammalian Cells. The focus of studies during the past year has been on factors which influence the expression of malignant transformation in vitro. It was shown that transformation may be greatly enhanced by post-irradiation exposure to the phorbol ester promoting agent TPA, suggesting that the effect seen in the classical mouse skin experiments may have a more general basis in the mechanisms of carcinogenesis. Recently it was shown that transformation induced by X-rays, with or without enhancement by TPA, can be markedly suppressed by treatment with several inhibitors of protease enzymes. The appearance of protease activity is associated with the malignant transformation of mammalian cells, and it has been shown by others that TPA itself can induce protease activity in normal cells. In particular, it induces an enzyme called plasminogen activator which is thought to play a central role in malignant transformation and carcinogenesis. Recently it was found that exposure to either TPA or X-rays alone can induce plasminogen activator in the normal mouse 10T½ cells which are used in transformation studies.

In separate studies, the effects of fluorescent light exposure in C3H 10T½ cells were studied. It was found that fluorescent light will induce malignant transformation in these cells, and that the frequency of transformation induced is related to dose. An observed plateau in the curve of transformation frequency vs. fluorescent light dose correlates well with previous results obtained for ultraviolet light induced transformation in this cell line. The similarity of these two patterns of dose-response for transformation frequency suggests that similar molecular processes may be involved in the induction of malignant transformation by the two types of radiation.

Two new experimental techniques have been developed during the past year which suggest that, although the initiating event in carcinogenesis may be a mutation, there are other important factors which determine ultimate phenotypic transformation. Another technique has been developed that has certain advantages over the mouse 10T½ cell system, though it is less stable and subject to spontaneous transformation. Studies have been continued of cytogenic changes induced by radiation and the correlation of these with transformation and mutagenesis.

Genetic Susceptibility to Cancer. These studies involve an investigation of the radiation response of human diploid cells isolated from patients who appear to be genetically predisposed to the development of spontaneous and/or radiation-induced cancer. Research during the past year has concentrated in three areas: Retinoblastoma, Bloom's Syndrome, and the study of certain cancer-prone families. It was found that cells from patients with hereditary retinoblastoma are significantly more sensitive to the lethal effects of radiation than are cells from normal individuals or patients with the sporadic form of retinoblastoma. Hereditary retinoblastoma is characteristically bilateral and multi-focal in origin, and is associated with an increased incidence of cancers at other sites and of cancer within the irradiated field following X-ray therapy.

DNA Repair. Research into the development of new techniques for measuring DNA repair capacity in mammalian cells has been continued during the past year. These include the host-cell reactivation assay of irradiated herpes virus which can be adapted to study of recombinational events in mammalian cells. The alkaline elution assay has been used to examine the induction of DNA cross-linking by various chemical agents. N-acetoxy-AAF and DMBA were found to be particularly effective in inducing cross-links, whereas benzathracene, benzathracene hypoxide and MNNG were considerably less effective.

Environmental Toxicology, Inhalation. Research continues to be focused by the premise that most respiratory disease is either initiated by or at least complicated by the inhalation of particles and gases. Emphysema, bronchitis, pneumoconiosis, neoplasms, and infectious disease all may be consequent to inhalation of noxious particles, and the severity of the resultant disease may be influenced by the number of particles deposited as well as their site of deposition and their ultimate fate. This program represents an attempt to explore the diverse physiological mechanisms which prevent the accumulation and deleterious action of

inhaled particles and gases as well as the mechanisms involved in the pathogenesis of environmental lung disease.

Attempts are being made to understand important factors which influence aerosol deposition. Currently the effects of exercise, disease, species differences, breathing pattern, and gravity on the distribution of deposited aerosols are being studied.

Experiments on the effects of exercise on the uptake of particles and gases in the respiratory tract and on the responses of the lung continue. Studies have been completed which demonstrate that exercising animals get pulmonary edema at approximately one-third the levels of ozone when they are exercising in a rotating wire cage with a doubling of oxygen consumption. LC_{50} curves are similarly dramatically shifted to lower ozone levels during exercise. Experiments have also been completed with exercising hamsters, which show a clear relationship between total particle deposition and oxygen consumption. A special treadmill for hamsters has been constructed and is now in operation, along with equipment permitting the measurement of oxygen consumption during exercise and at rest. It was demonstrated also that the collection efficiency increases during exercise in hamsters. The effects of breathing pattern on aerosol deposition have been studied in dog lungs using a servo-controlled ventilator.

Environmental Toxicology, Biochemical. The new Toxicology Unit in the Center began to occupy its recently renovated facilities in December 1978. An initial period of several months was concerned with establishing functional laboratories and new faculty and staff recruitment. The initial research efforts have been in areas of cell biology and biochemistry related to environmental toxicology. The focus has been on the actions of tumor promoters (phorbol diesters) on plasma membrane-mediated functions on differentiated cells in culture and on the regulation of receptors for peptide regulatory and growth-controlling factors. These are fundamental investigations on the mechanisms by which normal and malignant cells control growth and differentiated function. They are directly relevant to an understanding of how certain environmental chemicals produce cellular toxicity and promote neoplastic transformation.

Occupational Respiratory Disease. A cohort of 850 individuals working in granite sheds exposed to silica is being studied. Dust exposure was previously related to the development of lung function abnormalities. Current activities consist of attempting to refine estimates of safe levels of exposure. These efforts involve further environmental sampling and estimation of dust exposure and further evaluation of the pulmonary function data. The study will also allow allocating risk to both dust exposure and cigarette smoking.

Neurological Studies. Di-methyl-amino proprionitrile was identified to be a neurotoxin in a population of workers exposed to this material used as a catalyst in the manufacture of urethane foam. Sixty-five percent of the population had urinary tract dysfunction. When the catalyst was removed from the work setting, most workers recovered. Clinical evaluation revealed that the toxic effect was neurologic in origin.

N-hexane is an occupational neurotoxin at high levels of exposure. The effect of chronic low level exposure are now known. Fifty workers exposed to this solvent in rubber manufacturing have been studied by standard neurological exams, nerve conduction studies and questionnaires. No differences were found between the group exposed to n-hexane and a comparison group not exposed.

Rates of DNA Damage. Sister chromatid exchanges (SCE) have been used as a measure of DNA damage in individual workers in a rubber factory. Workers who have the opportunity to be exposed to possible carcinogens have been compared to those known to be exposed to carcinogens. The rates of SCEs have not differed between these two groups. However, it has been revealed that there is a significant increase in the frequency of SCEs and cigarette smokers, both former and current as compared to non-smokers. Additional work populations exposed to potential carcinogens will be examined by this technique.

Community Air Pollution Studies. In preliminary findings of the "6-city" air pollution study, children were found to have significantly more respiratory infections and impaired pulmonary function in homes where gas was used for cooking and heating, compared to children in households where electricity was used. The probable reason is intermittent exposures to nitrogen dioxide at much higher levels indoors than occur in the ambient air outdoors. If confirmed in follow-up studies which are in progress, these findings have important policy implications to both general air pollution control and control of specific pollutants indoors.

Vanderbilt University

Although diverse in specific objectives, all current research projects in the Center are to some extent related to toxic substances found in the environment. Included as current research topics are toxic fungus and higher plant metabolites, pesticides, mutagenic, carcinogenic, and teratogenic agents from various sources and toxic metals.

Bio-organic Toxicology. The study of the biosynthesis of the toxic stress metabolites of the sweet potato has resulted in the isolation and synthesis of several new sesquiterpene metabolites from this plant. 6-oxo- and 6-hydroxy-dendrolasin are the least complex furanosesquiterpenes yet isolated from this source. The discovery of 9-oxygenated farnesols (position 6 in furanosesquiterpene numbering) has led to the postulate that side chain oxidation at this position occurs before furan ring formation.

Synthesis of the four sesquiterpenes have been developed which utilize 2-(2'-methylpropene-1-yl)-1,3-dithiane as a key intermediate. Synthesis of ^{14}C-labeled dithiane should allow labeling studies to determine incorporation efficiencies of the sesquiterpenes into ipomeamarone.

6-Hydroxydendrolasin and 9-hydroxyfarnesol are novel and have not been found in any other plant. 9-oxofarnesol has been isolated from the camphor tree and 6-oxodendrolasin has been isolated from South African plants. The biosynthetic pathway leading to these compounds may be general and apply not only to the sweet potato but also to a wide variety of other plants.

Toxicity studies were carried out on five of the 3-substituted furans that have been isolated from the sweet potato in recent years. The compounds were administered to mice I.P. to obtain LD_{50} values and to study the gross and microscopic pathology. The compounds studied (and the LD_{50} in millimoles per kg) were: ipomeamaronol (1.0±0.08), 6-myoporal (0.33±0.04), 4-hydroxymyoporone (0.88±0.32), 7-hydroxymyoporone (0.75±0.7), and dihydro-7-hydroxymyoprone (0.69±0.07). All the compounds produced temporary neurological effects. These effects were most pronounced with 7-hydroxymyoporone and dihydro-7-hydroxymyoporone. All the compounds caused the development of extensive necrosis in the liver. The LD_{50}'s of the compounds, except for 6-myoporol, are about the same as that for ipomeamarone.

Moniliformin is a highly toxic fungal metabolite produced by Fusarium moniliforme, a common fungal contaminant of grain. The compound is a vinylogous α-ketoacid and interferes with mitochondrial pyruvate and α-ketoglutarate oxidations. The interaction of moniliformin with thiamine was investigated to determine if thiamine destruction was responsible for the interference. It was found that moniliformin does not react with thiamine to form a compound which can be detected by NMR. It does not interfere with the non-enzymatic catalytic activity of thiamine and it does not combine with thiamine in such a way as to make it impermeable to rat brain cells. Moniliformin is an effective inhibitor of pyruvate dehydrogenase and transketolase from rat brain and liver.

Reactive Forms of Sulfur. Adaptations have been made of the known photochemical generation of singlet sulfur ($S(^1D)$) from carbonyl sulfide (COS) and of its insertion reaction with cyclohexane to give cyclohexanethiol. The thiol has been identified by gas chromatographic (gc) peak augmentation, mass spectra, and formation of the mercuric thiolate.

When the yield seems to be maximized, more effective traps will be sought through competitive reduction of the yield. Once the best trap has been selected, it will be used to seek non-photochemical reactions for producing $S(^1D)$. These new sources of $S(^1D)$ then will permit studying models of toxic reactions likely to occur between cytochrome P-450 and compounds in widespread use that can be hazardous because of C=S or P=S linkages.

In related work, a hydrodisulfide of the structure $HSSC(CH_3)_2CH(NHAc)CO_2CH_3$ is being sought because there is reason to believe it will be atypically stable and thus much more amenable to study than is usual for its class. With success, penicillamine derivatives may become the effective traps mentioned above, isolation of a pure hydrodisulfide may be unequivocally demonstrated by an insertion reaction of $S(^1D)$ with a thiol (confirming

beliefs for cytochrome P-450), and a model will become available for clarifying the behavior of the hydrodisulfide of cytochrome P-450, believed to be implicated in reactions of toxic C=S and P=S compounds.

Metabolic Toxicology. Multiple forms of rat liver microsomal epoxide hydratase were isolated and purified to apparent homogeneity. Three forms of the enzyme appear to be present in untreated rats as judged by amino acid composition, differences in isoelectric points, immunological criteria, and substrate specificity of the isolated proteins. After treatment of the rats with stilbene oxide or phenobarbital, one of the forms disappears; in the latter case, one of the major forms left may be altered. The isolated enzymes were compared to each other and to a number of epoxide hydratase preparations purified from human liver microsomes with regard to physical properties and reactivity with styrene oxide, 2-chloroethylene oxide, and five polycyclic aromatic hydrocarbon epoxides. The ability of liver alcohol dehydrogenase was utilized in devising a coupled spectrophotometric assay for epoxide hydratase that can be used for the assay of enzyme activity toward a variety of toxic epoxides. 2-Chloroethylene oxide, the epoxide derivative of vinyl chloride, was found to be a substrate for epoxide hydratase. Highly-purified epoxide hydratase and alcohol dehydrogenase were examined for their effects on covalent binding of label from ^{14}C-vinyl chloride. The results indicate that 2-chloroethylene oxide is formed (by cytochrome P-450) and rearranges to 2-chloroacetaldehyde; the latter compound appears to be a major alkylating species derived from vinyl chloride although it is less reactive than the epoxide.

Reproductive Toxicology. Mercury and cadmium have the potential for deleterious effects on the human fetus. Mercuric chloride, methylmercuric chloride and cadmium chloride directly effect the human placental syncytiotrophoblast microvillous membrane. These heavy metals alter the facilitated diffusion of α-aminoisobutyric acid (AIB) into vesicles of this membrane. Mercuric chloride induced an initial increase (27%) in AIB transport but, subsequently inhibited its uptake (33%) when compared to control. Methylmercuric chloride and cadmium chloride inhibited the initial rate of AIB uptake, but did not effect the equilibrium value. These effects were concentration dependent. Methylmercuric chloride was more potent in inhibiting transport than cadmium chloride, 40% versus 21%, respectively. Methylmercuric chloride and cadmium chloride effects on AIB transport were observed with minimal preincubation with placental vesicles. However, preincubation was necessary for mercuric chloride-induced inhibition of AIB transport. Cysteine protects against mercuric chloride and methylmercuric chloride-induced effects on AIB transport but did not reverse their effects. Mercury and cadmium-induced placental toxicity result by interactions of these heavy metals with the placental plasma membranes.

Mercuric chloride, methylmercuric chloride, and cadmium chloride directly effect the human placental syncytiotrophoblast microvillous membrane. These heavy metal compounds alter the facilitated diffusion of α-aminoisobutyric acid (AIB) into vesicles of this membrane. Mercuric chloride induced an initial increase (27%) in AIB transport, but

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Biosynthesis of Slaframine. Slaframine is a mycotoxin produced by Rhizoctonia leguminicola when grown on legumes such as red clover. Biosynthetic experiments using deuterated pipecolic acids (known precursor) have been undertaken to determine the manner of introduction of the primary amino groups at the 6 position of slaframine. Previous studies have shown no loss of deuterium from 6,6-d_2 pipecolic acid, ruling out the involvement of a Δ^5-enamine. Perdeuteropipecolic acid has now been synthesized and fed. Preliminary results show a loss of two deuteriums, suggesting a direct unactivated attack at the 6 position of slaframine to yield, presumably, a 6-keto intermediate. Involvement of a Δ^6 intermediate would have resulted in a loss of three deuteriums. Perdeuteropipecolic acid with a higher deuterium incorporation has now been synthesized, and the synthesis of 5,5-d_2 pipecolic acid is in progress. Feeding those compounds should serve to further verify the above results.

Fungal growth and slaframine production have been studied. Slaframine yield appears to increase with different media as shown: second cutting red clover hay steep < first cutting red clover hay steep < red clover seed steep.

Progress has been made in development of a practical synthesis of slaframine. Separation of the steroisomers of 1-hydroxy-6-tosylamidooctahydroindolizine followed by detosylation and acetylation should not yield slaframine.

Heavy Metal Ion Toxicity and Chelation Therapy. Data have been collected on a number of aspects of heavy metal toxicity which bear directly on clinical usage of recommended chelating agents. Among these is what is probably the first comprehensive comparison of the relative efficacy of the five "best" chelating agents for acute mercury poisoning. In this study significant differences were found among these compounds. Previously

they have been considered to be more or less interchangeable. The order of effectiveness is 2,3 dimercaptopropanesulfurate = N-Acetyl-D, L penicillamine > D-penicillamine > 2,3 dimercaptosuccinic acid = BAL. This now furnishes standard compounds for therapy gains by which the performance of any newly synthesized ones can be measured.

In the search for new therapeutic agents for mercury, two different chemical types of compounds have been investigated. The first of these have been water soluble dithiocarbamates (NCS_2^-), which have synthesized several new examples. The best of these show some effect on mercury poisoning but they are nowhere near as good as the sulfhydryl containing compounds listed above. The second type was the water soluble phosphines as exemplified in $\phi_2P\phi SO_3Na$. This compound also showed some slight ability to offset the toxicity of mercuric chloride but its own inherent toxicity was too great to allow it to be used at the levels where it would react more completely with the mercury.

Human Toxicology. The utility of nude (Nu/Nu) mice are recipients of human tissue xenografts in the evaluation of potential human toxins is being investigated. Since ethical considerations create necessary but severe restrictions in the scope and magnitude of clinical research involving human objects, mammals bearing human tissue potentially susceptible to suspected chemical and/or biological toxins would provide a unique tool to the field of experimental toxicology. The approach has been made possible by the recent discovery of the mutant nude mouse and its extensive study revealing that the ability to transplant xenografts is due to its genetic lack of a thymus and the consequent failure of the cell mediated immune system. The functionality of xenografts was demonstrated in nude mice by successful amelioration of chemically-induced diabetes in the nude by peritoneal rat Islet transplants. Limited success has been achieved with liver xenografts while xenografts of human and rat skin can be successfully transplanted using split thickness graft beds.

Biochemical Toxicology. Studies were continued on the metabolism of thiono-sulfur containing compounds by the hepatic cytochrome P-450 containing monooxygenase systems. One such study involved the examination of the metabolism of the anti-thyroid agent methimazole. Methimazole has been reported to cause liver necrosis in some patients administered methimazole for treatment of thyrotoxicosis. The results of our studies indicated that methimazole is metabolized by the cytochrome P-450 containing monooxygenase system to two major products, 3-methyl-2-thiohydantion and N-methylimidazole. These studies also indicated that a portion of the sulfur atom of methimazole was released as atomic sulfur which became covalently bound to liver macromolecules. These data suggested that the covalent binding of atomic sulfur to cytochrome P-450 was the mechanism by which methimazole, administered *in vivo* or incubated with hepatic microsomes *in vitro*, causes an inhibition of hepatic cytochrome P-450 containing monooxygenase activity.

The metabolism of the hepatonecrotic and hepatocarcinogenic agent thioacetamide by rat liver microsomes and a reconstituted cytochrome P-450 containing monooxygenase system was also examined. The results of these studies indicated that thioacetamide is metabolized by the cytochrome P-450 containing monooxygenase system first to a monooxygenated product, thioacetamide-S-oxide which is then metabolized in a second cytochrome P-450 catalyzed reaction to a reactive intermediate which is believed to be thioacetamide-S-dioxide. The S-dioxide may covalently bind to tissue macromolecules, breakdown to thioacetamide, or, alternately, degrade to as yet unidentified polar products. The results of these and other studies suggest that the liver necrosis seen on administration of tioacetamide to rats and other species is the result of the cytochrome P-450 containing monooxygenase catalyzed metabolism of thioacetamide, first to thioacetamide-S-oxide and subsequently to thioacetamide-S-dioxide which covalently binds to liver macromolecules leading to the production of liver necrosis.

Investigations carried out in collaboration with pathologists at the College of Veterinary Medicine, University of Georgia, have depicted the microscopic evidence of bronchiolar cell necrosis in rats injected repeatedly with 4-ipomeanol, a principal lung toxin from the fungus-infected sweet potato. Both Clara cells and ciliated bronchiolar epithelium are sloughed into the bronchiolar lumen. Regeneration of the Clara cell appears to occur by way of two different progenitor types, one of which becomes a new Clara cell and the other differentiates into ciliated epithelium.

Considerable effort has been made to detect, isolate, and describe the respective agents responsible for leucoencephalomalacia of equines (ELEM) and toxic pulmonary edema of swine (SPE), both associated with moldy corn. The former condition is caused by a toxic principle formed by Fusarium moniliforme growing on corn, and similar moldy grain is responsible for the pig disease. Laboratory animals do not respond to feedings of the incriminated feed samples or to concentrated solvent extracts of the corn. The possible role, if any, for the mycotoxin moniliformin, from F. moniliforme, in ELEM is not clear, and attempts to produce SPE by feeding corn cultures of F. moniliforme and of moniliformin itself have not been productive. A new metabolite of ELEM-positive strains of F. moniliform, a phenolic compound, was isolated and is currently being tested for toxicity in both laboratory animals and in a donkey. At present, the causative agent(s) for both of these diseases remains elusive.

University of California at Berkeley

Ongoing projects in this center range from basic research in the area of nucleic acid metabolism to improvement of the widely used Salmonella mutagen testing system and the development of a human dosimeter to assess DNA damage. The major research objectives of the center include:

1. Development of sensitive methods to detect environmental mutagens.

2. Use of these methods to identify environmental mutagens likely to be a significant human health hazard.

3. Analysis of transport and enzymatic activation of mutagens in the cell.

4. Assessment of the mechanisms by which mutagens interact with DNA and other cellular elements.

5. Definition of pathways of DNA repair and other cellular mechanisms which protect DNA from the effects of mutagenic agents.

6. Development of a potency scale for environmental mutagens and carcinogens.

7. Elucidation of fundamental biochemical mechanisms involved in the synthesis and degradation of nucleic acids.

8. Analysis of the effects of mutation on the structure and evolution of eukaryotic genes.

These ongoing projects can be roughly divided into four areas: Mutagenesis; DNA Repair; Nucleic Acid Metabolism; and Changes in Eukaryotic Gene Structure (and Evolution). Recent progress in each of these areas is reported here.

Mutagenesis. This field is the most active area of research in the center, involving projects in all of the member laboratories. Recent work in the Ames laboratory has involved improvements in the Salmonella test, development of methods to assess DNA damage in individual humans, establishment of a comprehensive data base on carcinogenic potency, and the detection of a number of new environmental mutagens.

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 reactivity of one of these classes may be due to the fact that the active forms of the carcinogens have very short half-lives. To facilitate the interactions of these active forms with the test bacteria, the Ames group has developed 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. An understanding of carcinogenic potency is the key issue in human risk assessment at the present time because of the large number of carcinogens-mutagens in the environment and the need to set priorities. A considerable amount of time has been devoted to developing just such a theoretical analysis and a great deal of time has been spent analyzing published cancer tests to develop a reliable carcinogenic potency data base. This data base on animal cancer tests includes over 2000 published cancer tests. The Ames group can now analyze this data base and ask a number of key questions in human risk assessment. They also can provide a calibration scale for examining the question of how much potency in short-term tests correlates with potency in animal cancer tests.

Although the primary focus of the Ames lab has been to develop new methods for analyzing the actions of mutagens and carcinogens, they have identified several new mutagens in the process of improving the test system. These include toxaphene and a new class of mutagens which are light activated. This latter class of mutagens may be particularly important in the development of cataracts and skin lesions.

In addition to mutagens of the type which interact directly with DNA, there are also others which act by interfering with cell division in various ways. Some work is directed at understanding basic mechanisms involved in cell division and the ways in which chemicals can affect this process. Workers have recently developed a method for isolating mitotic apparatus from embryos in a manner that allows retention of structure and certain essential functions including the energy dependent sequestration of Ca^{++} into vesicles associated with the isolated apparatus. Other studies involve biochemical analysis of the microtubules which make up the spindle fibers. In these studies, they have recently resolved two forms of β-tubulin and have explored the binding of GTP to tubulins, demonstrating considerable exchange of this nucleotide (60%) at a site which was previously considered "non-exchangeable." It seems likely that the two-pronged approach to this problem by definition of components and isolation of a functional apparatus will soon allow a detailed analysis of the mode of action of carcinogens, such as griseofulvin, which interfere with mitosis.

Other research is aimed at understanding cell growth and cell division in the unicellular eukaryote, Saccharomyces cerevisiae. A new method has been developed for studying cell surface growth and secretion by selecting mutants defective in the export of secretory and membrane enzymes. Genetic analyses indicate that at least 23 genes are responsible for the movement of these molecules to the surface. A number of mutants defective in chitin synthesis has also been isolated. These studies of the synthesis and deposition of chitin could lead to the discovery of novel ways to design selective and non-mutagenic pesticides since chitin is an essential component of insects and fungi but is not found in mammals.

Mutagenic agents can exert their effects directly on DNA nucleotides or they can cause mutations by inducing a process known as error-prone or "SOS" DNA repair. Recent studies have shown that this process may be mediated by an altered form of DNA polymerase which is observed in cells only after induction of the error-prone synthesis. Members of the laboratory are now determining the nature of the alteration in this enzyme and measuring the frequency with which it makes errors in the replication of defined template DNAs.

DNA Repair. Other members of the center are engaged in a biochemical dissection of DNA repair in both prokaryotes and eukaryotes. In the recent past, three major new DNA repair pathways have been characterized. Two involve new DNA repair endonucleases of E. coli, endonuclease III and endonuclease V. The former appears to recognize particular types of damage in products brought about by radiation or chemicals; the latter appears to recognize damage in the DNA by virtue of distortion and/or uracil content. The third, and perhaps most unusual, involves the joint action of endonuclease VI and endonuclease III to remove apurinic residues from DNA.

In addition, several human repair enzymes have been characterized, including apurinic (AP) endonuclease, UV endonuclease, uracil DNA-glycosylase, and an enzyme that specifically inserts purines into apurinic sites in DNA ("purine insertase"). This latter enzyme has been shown to be inhibited by caffeine--an important finding. It was also shown that xeroderma pigmentosum strains of complementation groups A and D are lacking an AP endonuclease isozyme with a high affinity for AP sites.

Finally, a permeabilized human fibroblast system has been developed that allows for the screening of agents that bring about DNA repair as well as for study of the DNA repair process.

A study of the repair of SV40 viral DNA in normal and xeroderma pigmentosum fibroblasts has confirmed the relative inability of the XP lines to repair UV damage and has shown that XP lines A and D are abnormally proficient in the reactivation of depurinated transfecting SV40 DNA.

Nucleic Acid Metabolism. Extremely sensitive methods for electrophoresis of RNases in RNA-cast SDS polyacrylamide gels and for activity staining of resolved, renatured RNases have been developed. These methods have been used to visualize, analyze, and compare the RNases of human serum, urine, cerebrospinal fluid, and leukocytes. In the process, the existence, in serum and in urine, of SDS and mercaptoethanol stable, pancreatic-like RNases of unprecedentedly high molecular weight have been documented. One of these (a 30,500 dalton protein from urine) has been purified to near homogeneity. Also, the often-reported elevation of total serum RNase activity in cancer patients has been confirmed and shown to be due primarily to increased levels of electrophoretic species observed in normal serum rather than to "abnormal" species derived from tumor tissue.

Eukaryotic Gene Structure and the Role of Higher Order "Mutations" in Evolution. Interest in understanding the role of mutations in the evolutionary process has continued. Point mutations in structural genes, along with regulatory mutations and mutations causing rearrangement of genes have been studied. The two gene systems are mitochondrial DNA and globin genes in primate series. In the past year or so, purified mitochondrial DNA from four species of primates have been produced and digested with 11 different restriction endonucleases. From the differences in restriction "maps" produced, it was possible to calculate an overall mutation rate for mitochondrial DNA, obtaining a value which indicates that this genetic material is collecting mutations at a rate ten times as high as the nuclear genome of the same organisms.

Using the DNA transfer and hybridization techniques developed by Southern, five ape species were mapped by α and β globin genes and compared to those of humans. In the course of these studies, it was found that the globin gene region has been highly unstable in primate evolution. The mechanism most likely to account for this rapid evolution of α genes seems to be unequal crossing over. Wilson and his collaborators believe that intron length, intergenic distance between duplicate genes, and differential regulatory elements between duplicates are affecting the probability of unequal crossing over. In a related study, the nucleotide sequence of the globin cDNAs of four primate species was determined and compared with published sequences for human, rabbit, and mouse. Changes in non-coding sequences are three times as frequent as those in coding sequences.

Finally, it was found that Old World monkeys possess a δ globin gene which is not expressed as it is in New World monkeys, humans, and apes. This finding implies that some sort of regulatory mutation must have occurred to eliminate the expression of this gene in the Old World monkeys.

University of Rochester

Dose-Response Relationships and Pharmacokinetics in Man. Thirty-two infants, prenatally exposed to methylmercury, and their mothers, were examined over a five-year period. Exposure of the mothers to methylmercury was caused by the consumption of homemade bread prepared from wheat treated with a mercury fungicide. The period of exposure was about one to three months.

The severity of poisoning in the mother was related to the maximum blood concentration (as determined by longitudinal hair analysis). In the infant, the severity of prenatal poisoning was related to the maximum maternal blood concentrations during pregnancy. Several cases of severe cerebral palsy were found. However, milder cases previously not identified in other studies are reported. The syndrome consists of varying degrees of developmental retardation in addition to exaggerated tendon reflex.

Dose-response studies in adult humans, exposed in the same outbreak, are also in progress. Statistical models will take into account not only maximum methylmercury concentration in blood but also the period of elevated concentration.

Observations in five volunteers consuming methylmercury in fish have allowed us to estimate the relationship between long-term daily intake and the steady state blood concentrations. Studies are also in progress in volunteers receiving single tracer doses of radioactive methyl or inorganic mercury salts. Preliminary observations indicate mercury is exhaled in expired air and that the inhalation processes are stimulated by moderate oral doses of ethanol.

Several types of procedures are being tested for enhancing the removal of heavy metals from the body. One of the most successful involves an application of hemodialysis in which diffusible complexing agent is infused into the blood stream entering the dialysis unit.

Inhalation Toxicology. Inhalation toxicology studies under this project have three main components: investigation of lead in human and animal subjects, evaluation of macrophage functions in rats and the metabolism of inhaled mercury vapor. Both types of investigations seek basic information on how toxic materials, e.g., heavy metals, affect the function of the lungs as an asborption site; they also research specific problem areas, e.g., host defense mechanisms and translocation kinetics.

Human and Animal Studies: Lead. No quantitative data on the pulmonary absorption of different physical and chemical forms of lead have been reported. Therefore a study to obtain such data was undertaken in human subjects following controlled inhalation exposures. Two well-characterized forms of lead were selected, viz., $PbCl_2$ and $Pb(OH)_2$, as surrogates for monomeric and polymeric lead found in the atmosphere. Exposures involved microgram to nanogram quantities of Pb and the uptake and redistribution processes were followed by virtue of the inclusion or radioactive Pb-203 in the lead aerosols. Detailed analysis of the data from 16 subjects are underway. Preliminary results indicate that within the boundary conditions set by nanograms of monomeric Pb, and micrograms of polymeric Pb, there are only small distinctions in the pulmonary absorption. The objective of the investigation in dogs is to obtain a linear compartmental model for the distribution and excretion of lead-203. The models described in this report are based on experiments in which the activity of the isotope is measured in plasma, blood cells, and excreta after the injection of lead-203 intravenously in the dogs. Details of the distribution of lead between compartments of the blood is being examined in vitro experiments in which the isotope is added to whole blood.

Genetic, Reproductive and Developmental Toxicology. Some non-essential metals are highly embryotoxic and some have been shown to be teratogens in mammals, including man. Yet, surprisingly little information is available concerning prenatal and early postnatal effects to aid in assessing the risk of increasing exposure to these industrial and environmental pollutants. A mouse model system is being used to simulate human

prenatal and postnatal exposure to methylmercury, other mercury compounds, lead, and other heavy metals with the expectation that such studies will aid significantly in examining and interpreting less extensive human data. The overall objective is to provide information which will aid in evaluating hazards to exposed human populations. Current and projected studies include: a) maternal-fetal methylmercury exchange and relative distribution of methylmercury in different maternal and fetal organs, especially developing fetal brain; b) mother-to-newborn transmission of methylmercury and lead via milk during the suckling period; c) developmental changes in excretion of methylmercury, mercuric mercury and lead; d) effects of pregnancy and lactation on excretion of mercury and lead; e) development of techniques for detecting somatic cell mutation rates in human populations; f) development of an assay for detecting abnormalities of chromosome number and structure in mammalian ova.

Developmental Changes in Excretion of Mercury Compounds. Studies are continuing to define the mechanism(s) involved in large developmental differences in rates of excretion of mercury compounds discovered in our laboratory. After exposure to methylmercury, preweanling mice excrete very little mercury until the 16th to 18th postnatal day when there is an abrupt increase of fecal mercury excretion to adult rates of elimination. Results to date provide strong evidence that the developmental change in absolute rates of fecal excretion is mainly the result of an abrupt increase in rate of demethylation at approximately the time of weaning. Current studies are aimed at distinguishing the relative importance of demethylation by gastrointestinal tract microflora compared to demethylation in the body proper. It is believed that the sudden developmental change in excretion rate is a very useful tool for defining mechanisms of mercury excretion. Observations should be of direct relevance in evaluating hazards of methylmercury exposure in human populations.

Effects of Diet on Methylmercury Metabolism and Excretion. It was discovered that marked changes in rates of excretion of mercury after exposure to methylmercury can be produced by placing mice on different diets. During the past year, studies have been extended of dietary effects on mercury excretion after animals have been exposed to mercuric mercury compared to animals exposed to methylmercury. Different diets result in minimal effects of mercury excretion after exposure to mercuric mercury compared to the marked effects which have been observed on mercury excretion after methylmercury exposure. Current studies are aimed at determining whether changes in diet alter gastrointestinal microflora so that the organisms with greater demethylating capacity are favored.

Effects of Lactation and Suckling of Young on Mercury and Lead Excretion. It was observed that mercury body burdens of mice who are suckling their young decreased significantly more rapidly than do mercury body burdens of non-lactating mice. These differences in rates of reduction of maternal mercury body burdens cannot be explained in terms of the amounts of mercury secreted in milk. It was also observed that there are large differences in lead elimination in lactating compared to non-lactating mice. In contrast to the findings described for methylmercury secretion

in milk, it has been determined that a significant fraction (25%) of the initial maternal dose is transferred to suckling pups during the first few days. Current studies are designed to determine the relative importance of hormonal factors and changes in gastrointestinal microflora on excretion of mercury and lead in feces and secretion of mercury and lead in milk.

Detection of Adverse Reproductive and Chromosomal Effects of Chemical Agents. Cytogenetic procedures were developed in a mouse model system designed to detect aneuploidy that has been experimentally induced to occur in oocytes after either the first or the second meiotic division. Screening is accomplished in freshly ovulated oocytes or in two-celled embryos to permit observations of chromosome abnormalities before the occurrence of death of embryo cells as a consequence of abnormality of chromosomes. A significant increase in the incidence of hypoploidy was found in cytogenetic studies of oocytes after experimentally delayed ovulation. Studies on two-celled embryos are now in progress. Delayed ovulation also caused retarded development in 3 1/2 day embryos. Projects have been designed to assess reproductive chromosomal damage in adult mice resulting from exposure before birth to methylmercury and lead.

Metal Interactions. The gastrointestinal absorption of cadmium was studied in animals as well as man. In rats the absorption ranged between 0.5-1% of the dose, whereas in man it ranged between 1-3%. Somewhat higher absorption was noted in female subjects who were iron deficient. Retention of cadmium in whole body was relatively long. The kidney appeared to be the organ that accumulated cadmium.

Studies were also carried out on the metabolism of Hg-metallothionein. The protein, when injected intravenously, was taken up by kidneys and at higher dose levels was also excreted in urine. In general, the metabolism of the Hg-metallothionein was similar to that of Cd-metallothionein, indicating that the fate of the protein was not determined by the metal ion.

A radioimmunoassay to measure metallothionein has been developed. The method is quite sensitive and can detect nanogram quantities of the protein. The procedure is expected to be a useful tool in screening cadmium-exposed workers and excessively exposed populations.

Biochemical and Physiological Toxicology. The success of toxicologists in defining a harmless level of methylmercury in food (or, conversely, in defining a non-threshold quantitative relation of exposure to damage) will depend in part on discovering the molecule that is responsible for the selective susceptibility of target cells to methylmercury poisoning. A corollary objective is to discover treatments that may block toxic actions or reverse the degenerative processes caused by poisoning.

Methylmercury poisoning causes highly selective damage to target sites in the brain, in spinal ganglia, and in peripheral myoneural junctions. Previous work at this center identified the acetylcholine receptor (AChR) as the target molecule for peripheral damage. The mechanism of the critical damage to the brain, however, remained undiscovered. It

was concealed, paradoxically, not by failure to find potential target molecules, but rather by the undiscriminating reactivity of mercurials with sulfhydryl groups present both on proteins and on low molecular weight ligands in living cells.

Which one of the numerous ligands was the most susceptible to inhibition by methylmercury? Previous research in this Program yielded evidence that this question could not be answered by means of conventional assays of concentration-effect relations. Efforts were made to find new techniques of assay and new theoretical models of dose-effect relations, specifically applicable to mercuric and organo-mercuric ions. The inhibition of calcium transport was chosen as the experimental basis for this work.

Efforts were continued to dissect the "calcium pump" into functional components, only one of which was susceptible to inhibition by methylmercury. The components were a calcium ionophore, an ion gate, a transmembrane channel, and an ATP bydrolytic site containing the site of inhibition. A technical advance was scored in the assay of calfiphorin (a mitochondrial calcium carrier discovered here in 1977): fully purified calciphorin was shown to work as a selective ion carrier when coupled with a synthetic cofactor, thus eliminating the non-specific effects of membrane lipids.

A new "inhibitor partition" model was validated experimentally with a demonstration that calcium transport was blocked by a one-hit mechanism, and that methylmercury had a selectively high affinity to the inhibitory site of the calcium pump. This work is expected to lead to a new family of models of inhibition of enzymes by mercurials.

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Metabolic and Molecular Effects of Various Chemicals. It has been demonstrated that in the presence of the appropriate cofactors, carbaryl will bind to rat liver microsomal proteins *in vitro*. Experiments with different enhancers and inhibitors would support the hypothesis that binding is primarily to nucleophilic sites on the microsomal protein. Covalent binding could also be demonstrated *in vivo* with the small intestine, liver, stomach and kidney being the tissues most actively involved. The addition of carbaryl to liver microsomal systems inhibited the activity of certain monooxygenases. When the compound was administered *in vivo*, an enhancement of the activity of these enzymes was observed.

Naphthol, a hydrolytic derivative of carbaryl, was also bound covalently to the proteins of liver microsomes. Nitrosocarbaryl, a compound which can be derived from carbaryl in the presence of nitrite, is also being investigated in these systems.

It has been demonstrated that hexochlorophene will also bind covalently to the amino acid residues of hepatic microsomal proteins. Sulfur-containing amino acids seemed to be involved.

No metabolically-activated covalent binding to liver microsomal protein was observed with hexachlorobenzene in vitro. Some binding was observed when the compound was administered in vivo with the most extensive binding being observed in the spleen and liver.

Hedroxychlorodiphenyl ethers, contaminants of commercial pentachlorophenol, are potent hemolytic agents. At low concentrations these compounds can also protect against hypotonic hemolysis, induce prelytic efflux of potassium ion from intact erythrocytes, and produce morphological changes. In these studies with human erythrocytes, the activity varies with the degree of chlorination, and to some extent, with the position of the hydroxyl group. Hemolytic activity was not correlated with pKa. The most active compound is the 2-hydroxynonachlorodiphenyl ether. These compounds tend to be more active than the hydroxy-PCB derivatives tested previously.

The effect of a number of phenolic compounds, particularly the phenolic diphenyl ethers found as contaminants in pentachlorophenol, were evaluated for their effects and mechanisms of action on membranes. The hydroxy nonachlorodiphenyl ethers proved to be very potent hemolytic agents using human erythrocytes as the model membrane. Within the series of hydroxy chlorinated diphenyl ethers, the hemolytic potency varies with the degree of chlorination and to some extent, the position of the phenolic group. It was found at low concentrations, the substances could protect the erythrocytes against hypotonic hemolysis and do this at concentrations well below that of pure pentachlorophenol.

Experiments conducted in a pilot project have shown that several direct mutagens, such as dimethyl sulfate, bind to lambda phase DNA in sufficient quantities as to block cleavage sites for the restriction endonuclease EcoRl. Normally, six electrophoretically separable fragments are generated by cleavage of lambda DNA at five sites by the EcoRl enzyme. After treatment with various alkylating agents and gel electrophoresis, however, three new higher molecular weight fragment bands were seen in addition to the six fragments from molecules not being blocked at the binding sites. These results were consistent with the blockage at one restriction site and after long-term incubation with the alkylating agents blocking two sites.

The pharmacodynamics of several chemicals has been studied using benzene, phenol, and elemental mercury. Closed systems were used for benzene, and after a rat was introduced into the chamber the reduction in benzene concentration was monitored. The rate of benzene absorption decreased with increasing concentration of benzene in the atmosphere. Under these conditions, tissue concentrations reached constant levels within 30 minutes and the average half-life of the benezene in the tissues was 85 minutes.

Because phenol adsorbs to surfaces, the exposure of the rat was restricted to the nose. Phenol was rapidly absorbed through the lung and transported to other tissues.

When rats were exposed to air containing controlled concentrations of mercury, the tissue mercury levels increased steadily over a 3-day period. Highest concentrations were observed in lung and kidney.

Binding constants for four substituted phenols have been determined with serum albumin. These constants correlate highly with the blood-tissue ratios for liver and heart, but not kidney and lung.

The inhibitory effect of PCB (Arcolor 1254) on aflatoxin-induced carcinogenesis was studied and appears to be due to an effect on aflatoxin metabolism prior to tumor initiation rather than an effect on tumor growth and development. This conclusion is derived from the observation that when the aflatoxin and the PCB are fed together there is an inhibition of tumor development, while if the rainbow trout embryos are exposed to the aflatoxin for one hour (sufficient to produce a significant tumor incidence) and exposed to the PCB after this exposure, there is no effect on tumor incidence.

In previous experiments on the effects of pollutants on pathogenicity of infectious agents and on immunity it has been established that heavy metals such as cadmium and lead impair both the primary and secondary immune response. Several different approaches have been used to define the mechanism by which these metals act. Lead, cadmium, and methylmercury did not produce a significant alteratio of the response of lymphocytes in mixed lymphocyte cultures, suggesting that the cell mediated system is not responsible for the suppression of humoral immunity produced by these compounds.

An effect on secondary immune response would indicate a possible effect on the memory cells which are developed after the initial challenge and are responsible for the secondary response. The memory response of lymphocytes was affected by the higher dosage rates of lead, cadmium or methylmercury--with lead and methylmercury impairing response and cadmium stimulating response. No significant effect was observed at the lower dosages used.

Mitogenic proliferation is a cell mediated phenomenon and agents which would interfere with this process could consequently be classified as acting against the cell mediated mechanism. It was not possible to make any definitive conclusions concerning the effects of different metals in this system because of experimental variation.

There is some indication from preliminary studies that lead and cadmium, when fed together, tend to interact. The incidence of virally-induced mortality was consistent with what one might expect, there being no synergistic or antagonistic interaction between the two metals.

In efforts to develop statistical methodology for toxicological studies, new methods have been developed for testing goodness-of-fit in regression models for survival data. This analysis has led to a variety of tests which can be applied in different specialized situations. Another phase of the project has been concerned with the development of methodology

for the analysis of response time data, using a regression analysis where the basic form of the probability distribution of time-to-response is left completely free. The efficiency of this approach compared to the more conventional parametric models has been evaluated.

Chemodynamics of Environmental Chemicals. Soil-water distribution coefficients have been measured for a series of neutral organic compounds and expressed in terms of the soil organic matter. A high correlation between this soil organic matter-water distribution coefficient and aqueous solubility is observed. Analysis of the adsorption isotherms suggests that the distribution of the compound from water into the soil organic matter is due to a simple partitioning process, rather than a specific condensation on the soil organic matter surface.

In further studies of the rate of evaporation of organic compounds from dilute aqueous solution, the effect of concentration and stirring have been examined. Compounds with high Henry's law constants tend to evaporate at a faster rate than the water and deplete the interface concentration. A constant, α, is introduced into the rate expression to account for this effect. It has been demonstrated that values for α increase with stirring, reducing the depletion effect. Concentration does not affect α values with compounds of low Henry's law constants. However, with compounds with high Henry's constant, increasing concentration decreases the α value, the reason being that the surface concentration depletion is more rapidly restored when more solute molecules are available. A synthesis of these concepts allows a prediction of α from the Henry's law constant, and a subsequent prediction of evaporation of organic molecules from dilute aqueous solutions.

A general algorithm has been developed for estimating different parameters given the basic structural functions of a mathematical model. Point estimates, regression coefficients and Hessian matrix estimates, Eigenvalues and linear and non-linear terms are generated. The algorithm has been used to develop an improved experimental design for adsorption-desorption studies can be used in the refining of existing and preliminary pharmacokinetic models.

Studies of Pyrrolizidine Alkaloids. Pyrrolizidine alkaloids, in particular monocrotaline, appear to affect hepatic lipid secretion. In treated rats, palmitate-^{14}C accumulated in the liver as triglyceride rather than phospholipid. Liver homogenates from these animals also increased the incorporation of acetate into non-saponifiable lipid. It was demonstrated that the conversion of mevalonate to steroids was not affected. However, the conversion of acetate to mevalonate was enhanced, in particular the activity of the rate limiting enzyme, hydroxymethylglutaryl CoA-reductase.

To evaluate the significance of pyrrolizidine alkaloid transfer in milk, dried tansy ragwort was fed to lactating goats and the milk given either to two calves or to rats. After ingesting the milk for four months the calves showed some hepatocyte degeneration. Rats have also been fed the milk from the treated goats; however, the histological changes have not yet been established.

Pentachlorophenol. Large quantities of pentachlorophenol (PCP) is manufactured and used in the United States each year--approximately 50 million pounds annually. The technical product contains a number of contaminants in appreciable quantity, among them the chlorinated hydroxydiphenyl ethers. The identity, structure, and amount of many of these compounds in the technical product have not been established. Five different hydroxyoctachlorodiphenyl ethers were isolated and tentatively identified from technical PCP this year. Structural identification and quantification were achieved through use of electron impact mass spectrometry and electron capture negative ion chemical ionization mass spectrometry.

The Photochemistry of Organic Pollutants. The photodecomposition of the oxime carbamate, aldicarb, has been observed in acetonitrile and in dilute aqueous solution. In the latter solvent two products were observed while with acetonitrile, a complex mixture was produced, both with the sensitized and unsensitized reactions. Quantum yields have been calculated for the production of the different products obtained in the acetonitrile system and mechanisms have been postulated.

A Study of Polychlorinated Biphenyls and Other Toxicants in Human Cells Grown in Tissue Cultures. A human fibroblast tissue culture system was established and used to determine the effect of polychlorinated biphenyls (PCBs) on these cells. Following exposure, the cells were harvested and analyzed for lipids, proteins, and examined microscopically. PCBs were shown to alter phospholipid and triglceride snythesis in the cells. Cytoplasmic inclusions appeared as early as 20 to 30 minutes following exposure to higher concentrations and were present within 24 hours at some of the lower concentrations. Since phospholipids and glycerides are essential for maintenance for cellular integrity, these findings would indicate one of the basic mechanisms of action of PCBs.

Hydrazine. A method for measuring $^{15}N_2$ expired by rats following administration of ^{15}N-labeled substrates has been devised. Measurement sensitivity was below 10 µmoles $^{15}N_2$ produced over a 24-hour period. Studies of the metabolic disposition of ^{15}N-hydrazine indicated that over a 48-hour period about 25% of single 1 µmole/kg doses were converted to $^{15}N_2$. Higher and lower doses produced little change in that fraction. Urinary hydrazine accounted for almost 29% of the dose of 48 hours, and a hydrolyzable derivative(s) of hydrazine accounted for about 24%; total respiratory and urinary excretion accounted for about 75% of the dose. In the blood, both components were measurable for at least 24 hours. The derivative fraction is thought to include some mono- or diacetylhydrazine but identification has not been clearly established. Measurements during continuous infusion of hydrazine showed that at dose rates below 0.167 µmole/kg/hour, blood hydrazine usually reached a steady state proportional to input rate.

Protective Clothing for Pesticide Applicators. This project, designed to provide protective clothing for those who must handle and use chemicals, has benefited greatly by association with the Center. The project on Chemodynamics has been an invaluable source of information in designing the work and investigations of this particular project. The physical

chemical data provided on compounds and their behavior have been essential to conduct this particular project. The results to date have shown that by treatment of ordinary textiles of fluoroaliphatic resins, considerable repellancy to chemicals is achieved. Studies with animals as well as laboratory penetration studies have demonstrated the effectiveness of this treatment.

RESEARCH HIGHLIGHTS
Regular Research Grants Program

The Regular Research Grants Program includes approximately forty New Investigator Research Awards (R23) and sixteen Research Career Development Awards (K04). As indicated in the reports of prior years, the K04 grants represent a wide variety of research interests in environmental health sciences. These applicants continue to perform well as judged by the number and quality of the publications being reported.

Approximately one-half of the R23 projects are now in their last (03) year which is the time limit placed upon these projects. Reports are beginning to come in indicating conversions of these research programs to the regular research support mechanism. This conversion rate, as an indication of the attainment of the goals of the program, will be followed with interest.

The Extramural Research Program, for administrative purposes, is divided into two major research areas (1) prediction, detection and assessment of environmentally-induced diseases and disorders and (2) mechanism of environmental diseases and disorders. The percentage distribution of the Regular Research Grants Program is 52.5 assessment and 47.5 mechanism.

AIR POLLUTANTS AND RESPIRATORY DISEASE

Widespread environmental and occupational exposure to a variety of mineral dusts lend importance to studies promoting an understanding of the relationship of these agents to adverse health effects. Although serious human diseases result from exposure to toxic mineral particles, the mechanism of the pathogenesis of these disorders is not fully understood. One study is attempting to identify specific membrane lesions which are responsible for cell death due to silica in macrophages. Experiments to date show that cell death is dependent upon extracellular Ca^{2+} and may be mediated by an influx of these ions across the plasma membrane permeability barrier damaged directly by the toxins.

Models for the characterization of regional deposition of inhaled particles in humans are being developed in order to understand the interrelationships between airway configurations and size, and particle deposition. Such studies will lend credibility to the estimation of population risks associated with exposure to contaminated air.

Nitrogen dioxide, carbon monoxide and sulphur dioxide are also by-products of fuel combustion and are thus quite prevalent in the human environment. The mammalian toxicity and metabolism of sulphur dioxide is under investigation in several model species. Whether or not prenatal exposure to carbon monoxide causes any temporary or permanent behavioral effects, and if there is

a threshold for these effects, are also being determined. The effect of nitrogen dioxide on specific and non-specific pulmonary immune responses is under study as is its effect due to its oxidation potential. Similarly, the mechanistic basis for lung damage due to ozone, a major component of smog, is being determined.

Although a great deal of information is available concerning the effects of O_3 and NO_2 on respiratory tissues in animals and their relationship to acute respiratory infections, little is known concerning O_3 and NO_2 on chronic respiratory disease. Chronic respiratory disease is produced in pathogen-free mice by exposing them to Mycoplasma pulmonis, following which the animals are subjected to O_3 and NO_2. The effects of these gases on the chronic disease process are being evaluated by determining lung histology and the immune response of the animals.

An interesting approach to the study of the effects of SO_2 on intramural homeostasis utilizes New Castle disease virus (NDV) infection in chickens exposed to SO_2. Evidence of an effect of SO_2 at low levels on the spread of virus in the nasal mucosa was negative, although SO_2 was noted to cause a decrease in nasal mucociliary rates.

The interactions of bisulfite, the aqueous form of sulfur dioxide, with nucleic acids and various other cell components are being studied to elucidate the mechanisms whereby SO_2 exerts its effects. It appears that there is a reduction of the bisulfide to cysteine which concentrates in the cell and acts to shut down various metabolic processes by feedback inhibition.

Two studies are underway to provide insight into the association of environmental insult to lung tissue and collagen formation such as might occur in human emphysema and other forms of human pulmonary fibrosis. In one of these, a good correlation was found between the dose of ozone and degree of stimulation of collagen synthesis in the rat. Qualitative studies have identified more α1 (III) relative to α1 (I) solubilized from lungs of rats that were exposed to either paraquat or ozone. Another collagenous molecule, previously uncharacterized in lung tissue believed to be a basement membrane collagen similar to type IV, has also been observed.

Long-term NO_2 inhalation (30 ppm for 30 days) was suggestive of an increase in type I to type III collagen when compared to controls. When compared with controls, the relative proportions of types I and III were unaltered. Turnover studies of collagen and elastin, using radioactive tracers, suggest that there is increased degradation soon after NO_2 administration, while synthesis was only minimally affected.

The role of the lung in total body clearance of xenobiotic agents and the effects of exposure of the lung to exogenous agents is being determined. Rats exposed to 250 ppm CO 8/hour/day for six weeks exhibited no effects from such exposure on a variety of pulmonary parameters that were measured. The clearance of 5-hydroxy tryptamine (5HT) from the circulation depends upon metabolism by monoamine oxidase (MAO) and although the lung contains only eight percent of the MAO as in the liver, in rats, clearance of 5HT in the lung was three times the rate of clearance in the liver. These results point to the relative importance of the lung in clearance of xenobiotic agents.

A number of investigations are being supported to elucidate the relationship between air pollutants and human health effects in relatively stable population tracts. One such study relates mortality and morbidity to air pollution data. Those tracts with significant reduction in air pollution show greater decrease in total mortality rate for six of eight age-sex combinations studied. The validity and significance of these findings are being studied.

A major prospective epidemiological study of air pollution and respiratory disease is continuing in a six-city investigation. This study relates respiratory health effects to particulates and sulfur oxides. Both indoor and outdoor monitoring for SO_2 and NO_2 is a feature of the study.

CARCINOGENESIS

The NIEHS has an interest in and supports studies on all aspects of the interrelationship which exists between environmental agents and cellular macromolecules including metabolic activation, inactivation and interactions. One study using human liver cells is determining how malignant effects of chronic exposure to carcinogens present in the environment can be prevented or retarded by investigation of the enzymology of the excision repair of DNA damaged by model environmental carcinogens. Other investigations will elucidate the possible consequences to health of the reactions of ozone with organic compounds. These studies have shown that carbonyl oxides can act as a model for monooxygenase enzymes (MOX) and it is possible that the polycyclic aromatic hydrocarbon conversion to carcinogen can occur in polluted atmospheres in the absence of MOX where all of the other necessary reagents can be expected to be present.

Another study involving polycyclic aromatic hydrocarbons (PAH) is looking at the hypothesis that the mechanism of action of these compounds involves both initiation (mutation) and promotion stages analogous to the two-stage mechanism of cancer demonstrated for other types of agents. These studies involve examination of PAH or their non-mutagenic metabolites to determine whether they enhance mutation frequencies induced in V79 cells by mutagenic metabolites of PAHs.

An attempt is being made to understand the role of chromatin structure in relationship to the destruction of carcinogen-induced damage to DNA and subsequent removal and repair of the damage in human cells. Agents being studied include ultraviolet (UV) light and the chemical carcinogens N-acetoxy-2-acetylaminofluorine (NA-AAF) and 1-bromomethylbenz(a)athracene (BMBA).

The oxygen radicals hydrogen peroxide (H_2O_2), hydroxyl (OH) and superoxide (O_2^-) are generated by ionizing irradiation and by oxidation reduction cycles of quinones and hydroquinones of carcinogenic PAH. The mechanism by which these reduced oxygen species damage DNA/genetic apparatus is being studied. There is indication that DNA strand scission is induced by O_2^- and involves an intermediate in common with ionizing radiation, i.e., the OH ion.

The significance of the increase in polyamine biosynthesis that usually accompanies increased cellular proliferation in malignant cell growth is being assessed. The basic metabolic pathway involving ornithine decarboxylase and/or S-adenosylmethionine decarboxylase will be compared in normal cells, malignant cells and cells exposed to chemical carcinogens.

The N-nitrosamines are indirect-acting carcinogens which must be transported and metabolized for carcinogenic expression. The properties related to adsorption, transportation, metabolism, and reactivity with cellular macromolecules are all subjects of investigation relative to the carcinogenic potential of these compounds. Studies on the structure-function relationships indicate the importance of the role of adsorption and transport, as opposed to metabolic activities in non-carcinogenicity, as well as indications for the importance of structural features in certain nitrosamines. Such findings should provide a rationale for improving the predictability of short-term assays with regard to N-nitrosamines.

The carcinogenic potential of several substances of environmental significance is being investigated including cyclopenteno [cd] pyrene (CPEP). This compound is a widespread environmental and occupational contaminant and is found significantly in automobile exhausts. Preliminary studies indicate that CPEP is a potent carcinogen.

An investigation is underway to identify and quantitate the effects of risk factors in the environment using transformable cells in culture. The cell line in which the studies are being carried out are the mouse embryo C3H 10T½ CL8 fiberblast. This cell line undergoes malignant transformation when it is exposed under appropriate conditions to carcinogens such as photosensitive directives of fluorene. To assess the hazards in the enviornment, xenobiotics have been classified into primary or secondary risk factors. Primary risk factors are substances like benzo(a)pyrene, 2-acetylaminofluorene and aflatoxins which cause malignant transformation on their own. Secondary risk factors, on the other hand, are substances that do not cause cancer on their own but may interfere with the metabolic

activation or detoxification of the carcinogen. Studies to date indicate that the 10T½ cell line systems have not been sufficiently developed to detect primary risk factors; however, they may be potent detectors of agents that act as secondary risk factors.

The evidence seems conclusive that asbestos is hazardous at high dose levels but the dangers of low level exposure are not clear, nor is it clear what lung burden of asbestos places the patient in the high risk category. A study is underway to determine the source of asbestos exposure and the level of the exposure for various subgroups within an urban population. Moreover, an attempt will be made to correlate the level of total lung burden with the incidence of lung cancer in various populations. The present study will serve to describe the characteristics of fibers in patients with and without cancer taking into consideration age, smoking habits and occupation as closely as possible. The differences in trends will then be used to dictate the course of more exhaustive studies.

Bacterial mutagenicity and mammalian cell transformation studies were carried out on trichloroethylene oxide, cis and trans 1-chloropropene oxide, which are potential metabolites of their respective alkenes. Mutagenicity was assayed in S. typhimurium 1535 and E. coli WP2 strains. Genotoxicity was measured in E. coli A+/A- strains. Both the cis and trans 1-chloropropene oxide showed mutagenic or genotoxic activity in all three assays. Trichloroethylene oxide was not mutagenic in Salmonella but showed activity in the two E. coli assays.

Metal carcinogenesis continues to be investigated in a number of different aspects. In one study, it was shown that manganese exerted an anti-carcinogenic effect on nickel subsulfide and benzo(a)pyrene carcinogenesis and these findings are being followed up to provide further understanding of the molecular events that are associated with tumor initiation.

C-dimethylbenz (α) anthracene (DMBA), a potent tumorigenic compound is inhibited by the antioxidant BHT. In order to understand the events associated with tumorigenesis and inhibition of tumorigenesis, measurements of lipid peroxidation, peroxides, peroxidase, catalase, DNA, RNA and total protein of mammary parenchymal cells and liver from animals on different diets are being investigated with and without BHT.

BIOLOGICAL CONTROL OF INSECTS AND NATURAL TOXINS

The NIEHS continues to support a modest program dealing with the biological control of insects which is complimentary to a major interest in the health effects of pesticides. One such study has developed techniques which will be useful for the identification of baculoviruses and for quality control of the virus during their production for use in pest management. Another study dealing with the molecular biology of the granulosis virus of the Indian meal moth, Plodia interpunctella has resulted in the establishment of procedures for purifying granulosia virus free of all dectectable

host larval components, and to preserve viral structural integrity and biological infectivity.

In another aspect of a study on the biological control of insects, a significant effect has been shown for insecticide carriers on an animal host infected with viruses encountered by man. In this program, a number of commonly-used carriers have been studied using the mouse as an animal model and a variety of viruses as the infecting agent. Preliminary studies with influenza-type B Hong Kong strain virus suggests an effect of carrier on the synthesis of humoral antibody (immunosuppression).

The comparative aspects of toxicology relating to variations in the metabolic capabilities of different species of insects towards foreign compounds is receiving more attention. Such studies contribute to the knowledge required for the development of selective biocides such as insecticides.

Insects may also provide valuable models for studies elucidating the importance of mixed function oxidation. With these goals in mind, a program continues to attempt to establish the basic biochemical characteristics of the major catalytic components of the insect microsomal enzyme system and to study the interactions of the system with a variety of foreign compounds.

Mycotoxins are chemicals produced by molds which may be present in human or animal foods. Aflatoxin is a stable mycotoxin which has been demonstrated to be an extremely potent carcinogen. The mechanism of this carcinogenicity is being studied by a comparison of metabolic routes in two species of salmon. The effect of dietary modification on these metabolic routes is also being studied. Kidney effects have also been seen and the active metabolite in this process is being sought. Another common mycotoxin, rubratoxin B, is under toxicological investigation to determine its hazards and mechanism of action. Radio-labeled compound was used to determine tissue distribution and isolated liver perfusions were used to determine absorption, metabolism, and biliary excretion in that organ. Enzyme effects were also noted.

Other naturally-occurring toxicants which may be present in human or animal foodstuffs are being studied to determine how they cause their effects. Structure/activity relationships for a class of compounds (3-substituted furans) present on damaged sweet potatoes are being investigated. The biosynthesis of this and other "stress" metabolites is being studied in hope of learning how to prevent the formation of these toxic products.

The "Red Tide" has been a recurring algae problem on the East Coast in recent years. These aquatic organisms produce toxins injurious

to marine organisms and humans. The nature of these toxins is under study. Isolation and characterization efforts have been funded to attempt to learn the chemical structure of the toxin(s). Also, the life cycle of the toxin-producing organism is being determined to learn if control measures are feasible.

MUTAGENESIS

Extramural support for research in environmental mutagenesis reflects the broad diversity of interests of the NIEHS extending from a very basic understanding of the molecular events which underlie the mutation process to the development and improvement of methods for the detection and monitoring of mutational events.

In one study to detect mutations induced by environmental pollutants, two plant cell suspension culture systems are being developed for genetic studies. A plating system was devised to allow for rapid selection of mutants using either cell system. One such mutation detected by this procedure involving the mutation of soybean to maltose utilization, is being used as a model system to study different mutagens.

Studies with similar goals, that is, development of a rapid assay for mutational events, utilizes human cells as the target cells and intact hepatocyte as the activating system. Such an assay combines the activation capability of liver with an end point of obvious significance to human risk.

In another study designed to develop reliable in vitro assay systems for environmental mutagens and promoters using Chinese hamster cells, four DNA repair mutants were characterized for their cytotoxicity to radiations and chemical mutagens, unscheduled DNA synthesis (UDS) and UV mutability.

A preliminary study gave evidence that aphidicolin, a tetracyclic diterpenoid, slightly but significantly inhibited DNA repair synthesis. These results favor the observation that DNA polymerase may be involved in both DNA replication and repair. Moreover, data from this laboratory implicate that saccharin is a tumor promoter in animals. Whether it is a promoter for human beings is unknown. Similar findings of tumor promotion for DDT, but not mutagenic activity, per se, was also found.

A mutation dose response curve has been obtained using Chinese hamster ovary (CHO) cells exposed to UV light. The total number of UV-induced mutants obtained per loci screened was significantly greater that the control group. The number of shift mutants peaked at 10-20 percent of the survival dose. The number of mutants per locus in survivors was greater at this dose than at the higher dose. These results may indicate that UV induces mutations at the replicative fork during the S phase of the cell cycle.

A pattern of high correlation between carcinogenicity and mutagenicity has been demonstrated for chemicals in microbial assays systems. Improvement of the Salmonella/mammalian-microsome mutagenesis assay by introduction of a plasmid(s) superior to, or as a supplement to pKM101 may be capable of increasing the sensitivity and the reliability of the test system. In one such study, large numbers of plasmids are being screened in order to identify plasmids more effective than the pKM101, or one which can supplement the pKM101.

In order to assess the potential for various agents in the environment to produce a mutagenic or carcinogenic action, it is necessary to understand the nature of the alterations produced in the genetic material and the specific cellular responses which occur. The development of mutagen-sensitive Drosophila strains defective in precisely defined biochemical steps in DNA repair processes should provide tester strains for determining the mutagenic capability of various environmental agents and the mechanisms of action of agents found to have significant activity. The various parameters involved in providing basic information relative to these assay systems are underway.

A series of waxy mutants of known mutation origin in barley are being examined for use as an eukaryotic mutagen monitoring system. Such a system might be used to reveal very low levels of genetic damage and to record the nature of mutations induced at the DNA level. To date, spontaneous reversion frequencies of 17 waxy mutants induced by sodium azide and gamma rays have been determined. Reversion frequencies ranged from 6×10^5 to 32×10^5 for 1979 and 1980 and correlated well. Based on the data, four mutant alleles have been selected for further tests as possible in situ monitors. Additional mutants are being sought among M_1 spikes from N-methyl-N-nitrosourea, EMS, and gamma-ray treatments.

Drosophila melanogaster is providing a multicellular model system for the study of the genetic control of molecular processes involved in DNA repair in eukaryotes through examination of the biochemical defects associated with a number of genes which control mutagen sensitivity. Such a system will be employed for determining the mutagenic activity of various environmental agents and for studying the mechanisms involved in these activities.

Several carcinogens including diethyl sulfate (DES) and ethyl nitrosourea both of which ethylate DNA, are being studied to determine their effects on DNA and DNA replication in vitro. Also highly carcinogenic benzo(a)pyrene derivatives which produce a large polycyclic adduct on DNA were studied. Following treatment with these agents, measurements of spontaneous base loss and strand breakage in DNA were made.

Utilizing the Ames/Salmonella microsome test system, forty-six dyes, pigments and colorants are being examined for mutagenicity. These substances are used extensively in industry and have not previously been subjected to these kinds of tests.

A careful analysis of Dnase I resistant fragments at different expense of rearrangement indicates that initially the repair patch is not in close contact with core histones. Following rearrangement, many of the repair incorporated nucleotides are in core-like structures; however, complete randomization does not take place. Therefore, it appears that either the rapid rearrangement observed does not result in completely native core structures or following rearrangement repair synthesis is non-uniformly distributed in core DNA.

Another study is designed to develop and define two assay systems for screening mutagenic or carcinogenic potential of environmental agents. This system is a host-mediated assay for culturing mammalian cells especially human cells in diffusion chambers and subsequently implanted into mice. The induction of point mutations, chromosome aberrations, and sister chromatid exchange (SCE) of the target cells in the diffusion chambers will be used as indications for mutagenicity or carcinogenicity. The second system is a liver-homogenate-mediated assay using incubated diffusion chambers filled with rat liver extract. Cofactors and a compound under test with human cells in vitro using the same indicators as described for the first system are being used. These systems have been used to study eight different organophosphorus pesticides including methyl, parathion, demeton, trichlofon, dimethoate, methidathion, melathion, diazinon, and Di-Syston. The cultures are treated with the different organophosphorus pesticides at 10, 20, 40, or 80 micrograms per ml for two cell cycles and the incidence of SCE and extent of cell cycle delay was then analyzed. With the first four organophosphorus pesticides, a dose-dependent increase of SCEs were observed in cultures. A significant increase of such a chromatid exchange was observed only at the highest dose used for methidathion and medathion. The last two compounds did not cause any SCE increase at all the doses used. Thus, the two systems have been initiated and tested for screening mutagenicity and/or carcinogenicity of environmental compounds and have been found to be simple, economical in time and cost and of particular importance for compounds that need to be metabolically activated.

Another study has as its primary objective the development of a set of screening assays to test substances for their ability to induce errors in enzymatic DNA replication and to measure the ability of these assays for detecting altered reliability using DNA templates from tissues of animals exposed to carcinogens. Much of the research effort in this study so far has been devoted to the necessary preparation of analogue substrates suitable for use as probes of reliability, and the purification of standard enzyme and template solutions. The evidence that not only metallic carcinogens but also alkylating carcinogens enhance the incorporation of the arabinase analogue substrates is most encouraging. If indeed the mercuriated analogues and the seven metal DGTP behave in a similar manner, analogues for each of the four natural substrates would be available and the work could proceed with comparative tests for specificity and sensitivity in the detection of errors induced by direct acting carcinogens. Such assays will be helpful in detecting and classifying substances with unknown carcinogenic

potential. In addition, valuable information will be provided on the nature of the replication reaction itself, such as the kinetic consequences of geometric or charge-altered substrates capable of participating in the polymerase reaction.

A study to gain information on the basic mechanisms which lead to the inactivation or mutagenesis of mammalian cells by certain alkylating carcinogens-mutagens utilizes a highly synchronized Chinese hamster cell system in vitro in which the cells are treated with pulses of model carcinogens/mutagens. Using a model mutagen (ENU) quantitative dose response curves for mutagenesis and cell killing can be obtained in asynchronous CHO cells. The response in the cell cycle is relatively flat compared to other environmental mutagens such as x-rays and UV for the same type of synchronous CHO cells. It is believed, therefore, that the mammalian mutagenic response may be mutagen specific and also locus specific. Such would, of course, complicate mutagenesis studies in mammalian cells.

It is known that persons with the disease xeroderma pigmentosum have a nearly 100 percent incidence of skin cancer, and that the cells from these patients have a reduced ability to repair DNA damage. It is this correlation that suggests that even in normal individuals, the rate of DNA repair compared with other cellular processes is an important factor in the initial steps leading to carcinogenesis. It appears prudent to gain an understanding of the cellular repair processes in evaluating the carcinogenic hazard of humans to various chemical agents. One such study with this as its objective, is attempting to provide some understanding of the mechanisms of chromosome repair in mammalian cells and the relationship these processes may have to mutagenic and malignant processes. The approach being taken is to isolate CHO cells which are abnormally sensitive to killing by various mutagenic and carcinogenic agents, and to investigate the biochemical basis for their sensitivity. In order to investigate the role of DNA repair in the process of mutagenesis in mammalian cells, the mutation frequency at the hypoxanthine-guanine phosphoribosyltramsferase (HGPRT) locus was examined in the UV-1 and parental cells using the method of Hsia. Populations of cells were exposed to various concentrations of EMS, allowed to recover from the treatment and the frequency of clones resistant to the purine analogue six-thioguanine was determined. The results indicate that the UV-1 cell is three to four fold less mutable on a survival basis than the parental cell. These results suggest that post replication repair may be responsible for a large proportion of mutants seen in the mutagenized normal cells.

METALS

The NIEHS continues to support an important program on the health effects of metals commonly found in the environment. The spectrum of metals of interest is broad and includes selenium, cadmium, nickel,

mercury, lead, platinum, molydenum, arsenic, vanadium, zinc, cobalt, manganese and others, to a lesser extent. The scope of the research includes the identification of sources of pollution, conversion in the natural environment and in living organisms, catabolism and metabolism in various biological systems, effects on organs and organ systems, enzymes and proteins, and the pathological consequences involving neurological, developmental, behavioral, teratological, carcinogenic, mutagenic and embryopathic aspects. These studies on the metals range from the molecular, cellular and organ, to whole organisms including non-vertebrates, mammals up to and including primates and man. The NIEHS, in following the recommendation of the Task Force, will continue to focus its support of heavy metals research in areas of increasing environmental significance such as those occasioned by the inevitable massive increase in the use of fossil fuel predicted for the near future.

Among the more important metals with regard to human health effects is lead, an industrial and environmental contaminant arising from smelting and battery-producing operations, motor vehicle exhausts and lead-based paint found in many older homes. Although it is known that approximately 10 percent of ingested lead is absorbed, this is modifiable by nutritional state and dietary content. The role of iron, Vitamin D, season and other factors on the absorption, retention and excretion of lead is being studied, as is the pharmacokinetics (distribution) of ingested lead salts.

Lead affects principally three systems in man: renal, nervous and hematopoietic. The biochemical basis of the blood effects is under study to determine if prevention or treatments can be devised and whether the effects can cause other possible toxicities. The effects of low level lead exposure on the developing brain are being determined using rats as the experimental model. It is hoped that mechanistic and morphological observations can be correlated with behavioral and neurochemical disorders. Also, it has been found that lead exposure may cause toxicities to the autonomic nervous systen which express themselves in cardiac function aberrations.

Tests for exposure to lead are complicated due to dispositions of the material in bone. EDTA treatment which raises the blood lead level due to mobilization, may be useful in determining body burdens of the metal than straight blood sampling or urine collections. Exposure to zinc or cadmium may also affect test parameters currently being used to screen for excessive lead exposure. The extent of these effects is being determined.

On a cellular basis, a program is being supported to obtain fundamental data on the uptake and transport of lead and other metals and the effects of lead on lipid metabolism, cellular reproductive components and ultrastructural integrity.

Mercury exists in the environment due to natural erosion of rocks and soil and due to human activities such as industrial emissions and fuel combustion. Since inorganic mercury is relatively insoluble, it does not present more than a localized problem. However, the discovery that mercury can be methylated in the biosphere to form soluble and therefore absorbable organic mercury compounds, indicated the general environmental hazard of this metal.

It has been determined that mercury can influence the ion gradients necessary for proper cell function as well as alter the growth and development of the fetal brain. Furthermore, mercury can cause other fetotoxic effects through action on differentiating embryonic tissues. The nature of these effects is being studied by electron micrographs using diffusion techniques.

Another effect of acute mercury intoxication is interference with enzymes dependent on thiol group active sites. Chelation therapy to remove mercury has long been the standard treatment. An effort to develop new and safer compounds for this chelation has identified a number of possible agents for use individually or in combination. Different metals may interact to modify toxicity. For example, zinc seems to protect against cadmium toxicities as well as the hepatic toxicity of carbon tetrachloride. Studies indicate the mechanism for this protection is the linkage to NADPH in the drug-oxidizing system. Similarly, selenium, although toxic in higher doses, has been found to perform a role in heme metabolism. This role is under investigation to better understand the metal's beneficial and toxic effects.

Other examples of specific research projects involving the different metals include (1) neurotoxicity studies on low-lead exposure in developing rats using the parameters of brain acetylcholinesterase activity, amygdaloid seizures and potentials in the hippocampus, and electroshock seizure thresholds; (2) assessment of acute and persistent neurobehavioral effects of exposure to elevated but subclinical levels of lead early in life in monkeys; (3) an examination of the effects of perinatal lead exposure on brain growth, maturation and synaptic function; (4) a determination of the toxic effects of alkylmercury compounds in the intact animal in relationship to modifications in NA+, K+ATPase; (5) study of the embryopathic effects of chronic mercury intoxication on the primate; (6) induction of metal-binding proteins (metallothionein) and localization of metals in sub-cellular components; (7) assessment of the potential hazards to man from inhalation of trace metals at concentrations likely to occur in the environment; and (8) a study of the placental transport of organic mercury compounds and their potential teratogenic effects.

INDUSTRIAL AND AGRICULTURAL COMPOUNDS

Polychlorinated biphenyls (PCBs) are chemically inert, and therefore, environmentally-persistent compounds which are formulated for industrial uses. The major use is in the electrical industry as a dielectric fluid for capacitors and transformers. Current uses attempt to insure that no

environmental leakage occurs. However, prior uses and accidental releases have caused the compound to be rather ubiquitous in the environment. The toxic effects of low levels of PCBs on infant monkey behavior is under study. Mothers have been fed varying amounts of commercial PCB mixtures so that offsprings have been exposed both in utero and through mothers' milk. Significant hyperactivity has been found in all the PCB-exposed offsprings and preliminary indications are that some learning disabilities may also occur.

Elimination of PCBs from the body is very slow. The role that metabolism plays in elimination is not clearly established. Therefore, studies of the compound's influence on the hepatic mixed function oxidases and the effect of these enzymes on the compound are underway. Likewise, research to study the relative toxicities and metabolism of PCB isomers is being supported.

Liver damage is one of the common toxic effects of PCB exposure. A biological model, the chick embryo, is under study to test its validity to further define the biochemical basis for this type of damage. The model has already been determined to be reasonable using dose/response experiments. Histopathological effects on digestive tissues exposed to PCBs are being examined. The goal is to determine if the effects are focal, or general, and if cell reproduction is affected. Chlorinated hydrocarbons are common industrial solvents and reagents which may be present in the working environment. A determination is being made as to whether chronic inhalation of this substance by female rats causes embryo toxicity, behavioral defects, or cancer in offsprings. Carbon tetrachloride is known to be hepatotoxic, and the effect on the enzymes responsible for hepatic mitochondrial repair is being investigated, as well as its catabolism. Carbon tetrachloride lipid peroxidation is being studied as the possible toxigenic event. The metabolism of vic-dehaloalkanes to olefins is being mechanically defined in hopes of determining the decomposition pathways for these related compounds. The enzymatic dechlorination of small halogenated hydrocarbons is being studied to learn the reactive intermediates responsible for toxicity. Likewise, the mechanism by which haloalkenes, such as vinyl chloride, are activated to toxic metabolites is being studied. These transformations are also being related to liver lesions.

The liver toxicity of several chlorinated components is being studied using mammals and fish. TCDD produces a number of chlorinated functional changes. Serum enzyme and blood clearance tests have been developed which detect TCDD-induced hepatotoxicity in rats and rabbits.

Distribution studies and functional tests on acrylonitrile, an important industrial intermediate, are underway as well as the molecular interactions associated with its toxicity. Acrylamide is being studied similarly for its neurotoxic effect.

The basis for the carcinogenicity/mutagenicity of polycyclic hydrocarbons is being studied by fractionating metabolites by HPLC and assaying them in bacterial systems. For benzoflourourethane, the active mutagen appears to be the bay region diol epoxide. For polycyclics without bay regions such as cyclopentapyrene (CPP), the active compound appears to be CCP-3,4-oxide.

A polybrominated biphenyl mixture (Firemaster) accidently became mixed with animal feed in a section of Michigan several years ago causing widespread contamination of foods. The effect of the health and reproductive capacities of non-human primates is being carried out as a consequence of this incident. Menstrual cycle irregularities have been observed at levels of exposure permitted for humans. At higher doses, infants born to exposed mothers were smaller at birth, grew more slowly than normal and showed some behavioral abnormalities.

Dichlorodibenzidine, a widely used compound in the dye and polyurethane foam industries, is a potent carcinogen. The metabolic fate and mechanism of carcinogenicity of the compound are being studied using rats as a biological model.

Organophosphorous (OP) pesticides work by interfering with nerve transmission processes. This action is common in both the target species (insects) and man. However, it has recently been learned that delayed neurotoxicities also occur in mammals and present a significant and previously unknown hazard to man in his use of these pesticides. To learn the mechanism of this toxicity, a chronologic study of the histological changes in the sciatic nerve and branches, and the spinal cord of hens has been carried out. Degeneration was found to occur in the peripheral nerves first. Pharmacokinetics and pharmacodynamic parameters were also measured. The cat has also been found to be a useful model for this study. The precise biochemical nature of OP pesticides is also under investigation.

The enzyme most susceptible to, and responsible for, the toxicity of OP is being isolated for characterization studies. Correlations between inhibition and tissue toxicity are being made.

Insecticide synergists are being studied for their mechanism of action. It appears that some operate by inhibiting the P-450 oxidizing system.

The teratogenic effect of OP pesticides is being studied via fertile egg injections. A timetable of events relating agent administration to subsequent aberrant developmental reponse is being developed.

The biochemical mechanisms of Kepone-induced neurotoxicity are under investigation. Kepone, it appears, may act in the central nervous system by influencing membrane transport/GABA uptake. In addition, it has been determined that Kepone affects the secretory function of cells in the reproductive organs. This, in turn, may have a significant effect on the reproductive capacity of exposed species and on the health of their offsprings.

"Hexachlorobenzene" (HCB) is a widely-used pesticide which is quite persistent in the environment and little is known of the long-term effects of exposure to this chemical. It is known that animals exposed to HCB develop porphyria and the mechanism for this is being studied. This may also yield valuable information on how other chlorinated benzenes, including the PCBs, cause porphyria.

The herbicides paraquat and diquat are known to affect the lungs. In studies on the mechanisms of these effects on lung tissues, enzyme and other biochemical studies have shown an increase in ATPase activity prior to subcellular alterations observed through the microscope. Lung mitochondrial respiration was also affected.

In studies on the carbanate group of herbicides in mammalian cell culture, it was found that cytoplasmic microtubles were destroyed/ inhibited by two of these agents (CIPC and IPC). This effect appears to be generated through the cells' microtubule organizing centers.

Studies are being carried out to determine whether chronic, sub-lethal exposure to rats to carbamate and organophosphate cholinesterase (ChE) inhibitors significantly alters biochemical, physiological, histological or pharmacological parameters of the nervous system. Another investigation of the inhibition of NA-K ATPase by Kepone and dichlone, has shown that Kepone apparently inhibits binding to the inward facing sites of the enzyme with a different concentration dependence than its inhibition of binding, to the outward facing sites.

PHYSICAL FACTORS

Although the NIEHS concerns itself with the study of physical factors in the environment which produce undesirable health effects, that segment of the research program supported by grants continues to be small. Individual research projects relate to (1) photosensitivity, (2) noise, and (3) irradiation including microwave, ionizing and the biological effects of 60 hertz electrical fields.

Photosensitivity reactions of a reportable nature seem to be on the increase in the general population. Many of these adverse cutaneous events relate to exposure to chemical agents and light. One study designed to understand the molecular events which relate to exposure to photosensitizers such as chlorpromazine, anthracent and psoralen is underway. In the case of chlorpromazine, the cation radical formed upon irradiation was found to be not the lytic component and the active photoproduct does not require oxygen to produce its membrane-damaging effect.

Another commonly-used substance, musk ambrette, was shown to be photo-allergic but not photo-toxic in guinea pigs. Mild skin abrasion was required to demonstrate the sensitization, a condition which parallels the clinical picture in man.

In related preliminary clinical studies, patients with porphyria cutanea tarda were treated with plasmaphercsis and achieved remission, comparable with treatment by phylebodomy.

In the area of noise research, time-intensity trade off is a major concern of the national effort in consideration of the health effects of noise. The 3-dB rule (as intensity is increased by 3-dB, duration must be halved), or the 5-dB rule, are only accurate for a very restricted number of applications. In other instances, the rule could overestimate the likelihood of noise-induced hearing loss. Studies are underway to provide a more solid scientific basis for efforts in noise control and to develop a simple and accurate tool for assessing the risk of noise-induced hearing loss.

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